American Journal of Psychiatric Rehabilitation

Expert Opinion Article

Phytochemical Analysis, anti-inflammatory, analgesic and anti-spasmodic potential of *Isodon regusus* and *Phytolacca latbenia*

Ahmad Hassan Khan[1], Muhammad Adil[1,2]*, Muhammad Naseer[1,2], Sadia Iftikhar[3], Fatima Jahan[4], Faiza Tawab[4]

[1]Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology, Peshawar, Pakistan.
[2*]Centre for Plant Sciences and Biodiversity, University of Swat, Charbagh, Pakistan.
[3]Islamabad Model College for Girls
[4]Shaheed Benazir Bhutto Women University

***Corresponding author:** Muhammad Adil
*E-mail: adilbotany@qurtuba.edu.pk

Abstract
Herbal medication from natural products has analgesic, antispasmodic and antiinflammtory effects on human health. This research aimed to investigate these major activities along with the phytochemical analysis. Phytochemical analysis was carried out using a series of standard qualitative tests following established protocol to detect the presence of various bioactive compounds. The analgesic activity was evaluated using the hot plate method, analgesic activity was performed using charcoal meal test and antiinflammtory activity was determined using carrageenan-induced paw edema model in rat. Qualitative Phytochemical screening of the ethanolic and methanolic extracts of *I. rugosus* and *P. latbenia* revealed the presence of key secondary metabolites including alkaloids, flavonoids, phenols and tannins. Quantitative analysis indicated that tannins are the most abundant phytochemical in *I. rugosus* with concentration of 79.41% in the ethanolic extract and 51.39% in the methanolic extract. Whereas, *P. latbenia* exhibited the highest concentration of alkaloids, 81.36% in the ethanolic extract and 73.70% in the methanolic extract. The analgesic potential of *I. rugosus* were assessed at doses of 100, 200 and 300mg/kg, where the ethanolic extract demonstrated a maximum decrease in latency time of (27.45%) followed by methanolic extract (24.30%) after 90minutes dose of 300mg/kg. Similarly the methanolic extract of *P. latbenia* showed the highest analgesic potential (51.94%) followed by methanolic extract with a 48.36% after 90 minutes at a dose of 300mg/kg. The antispasmodic activity of *I. rugosus* exhibited maximum percent inhibition of 68.34% in the ethanolic extract and 65.00% in the methanolic extract at a dose of 300mg/kg after 90minute. In contrast *P. latbenia* showed a maximum inhibition 51.74% in the ethanolic extract and 47.60% in the methanolic extract at same dose and interval of time. The anti-inflammatory activity of *I. rugosus* showed maximum percent decrease in paw volume (34.54%) in the ethanolic extract and (30.00%) in the methanolic extract after 90minute at a dose of 300mg/kg, while, *P. latbenia* exhibited high percent decrease in paw volume (40.59%) in the methanolic extract followed by ethanolic extract (28.71%) at a dose of 300mg/kg after 90 minutes. it was concluded that these both plants having the analgesic, antispasmodic and antiinflammtory potential due to the presence of various phytochemicals.

Key words: *Isodon regusus* and *Phytolacca latbenia*, phytochemical analysis, analgesic, antispasmodic and antiinflammtory.

Introduction

Plants as autotrophic organisms exhibits two type of metabolism; primary metabolism, which is essential for basic cellular functions and is present in all living organisms and secondary metabolism, which enable plants to produced specialized chemical compounds (Mendoza and Silva, 2018). Medicinal plants have been used for centuries across the world to treat various diseases, owing to their richness in bioactive phytochemicals such as alkaloids, tannins, flavonoids and phenolic compounds, all of which possess significant therapeutic potential (Revathi *et al.,* 2024). Primary metabolites including amino acids, nucleotides, lipids and sugar are essential for the normal growth and development of plants, while secondary metabolites are organic compounds which do, not exhibit a direct function in the development and growth of plant although possess suspected role in the protection of plants from infections (Adetunji *et al.*, 2020). Among these secondary metabolites phytochemical demonstrate diverse biological activity. For example, flavonoids exhibit anti-mutagenic, anti-inflammatory and anti-carcinogenic potential (Kumar & Pandey, 2013). Alkaloids contribute to the treatment of disorders by exerting anti-inflammatory and analgesic effects (Madaan *et al.,* 2012).Tannins are known for their protein-

binding ability, forming insoluble complexes that precipitate (Mueller-Hervay *et al*., 2019), while Terpenoids exhibit antiviral, antibacterial, antitumor and antiinflammtory properties (Sun and Liu, 2011). The presence of these phytoconstituents highlights the critical role of medicinal plants in modern pharmaceuticals (Nawaz *et al*., 2022).

Pain management is a critical aspect of surgical and clinical care. Numerous studies worldwide have explored optimal strategies for pain relief. Medicinal plants serve as a significant source of bioactive compounds with analgesic properties (Zare *et al*., 2018). Conventional analgesic drugs include paracetamole (acetaminophen), non-steroidal anti-inflammatory drugs (NSAIDs) like salicylate and opioids such as morphine and oxycodone, each having distinct mechanism and histories of applications to treat various type of pain across a diverse population (Rauf *et al*., 2017). Many medicinal plants are rich in phytochemicals with antispasmodic properties, offering relief from gastrointestinal pain and contraction. Herbal remedies are effective in treating conditions such as colic and diarrhea associated with gastrointestinal hyper motility. For example atropine extracted from *Atropa belladonna* serves as an anti-muscarinic agent, effectively treating gastrointestinal spasm triggered by acetylcholine (Akinmurele *et al*., 2023).

Anti-inflammatory drugs work by interrupting biological pathway of inflammation, reduces tissue damage and enhancing patient comfort. While non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to reduce the inflammation and pain. On other hand various herbs that exhibit medicinal potential possess anti-inflammatory pharmacological properties with minimal or no negative effect offering promising alternatives in the therapeutic intervention (Akhlaq *et al*., 2022).

Isodon rugosus Wall. ex Benth, a member of the family Lamiaceae, is commonly found in northern Pakistan as well in other countries of the sub continents. It is a branched shrub growing between 1-5ft in height with upright stem and ovate shape leaves covered in fine hairs with rough margins. It's flowering period span from July to September, with seedling development occurring from August to October (Sharifullah *et al.,* 2016; Sadiq *et al*., 2018a). Traditionally *I .rugosus* has been used for a variety of ailments including toothaches, as a bronchodilator, for managing diarrhea and as an antiseptic. Extract obtained from its fresh leaves are used to alleviate disorders such as Scabies, hypertension, fevers, rheumatism, toothaches and headaches. Various extracts and fractions derived from *I. rugosus* show the capability to combat bacteria and fungi in addition to exhibit antioxidant properties (Janbaz *et al*., 2014).

Phytolacca latbenia (Moq.)H.Walter locally known as "Challaha Kafal'' belong to the Phytolaccaceae family and is abundantly found in Pakistan (Shoab *et al*., 2017). *P. latbenia* is found across a wide area covering about 13,200 sq. km, altitude ranging from 1500–3000 m in the Murree, Galyat, Swat, Dir, Kaghan, and Kashmir hills (Ullah *et al*.,2015). It is used for Narcotic, purgative, antifungal, antiviral and anti-rheumatic purposes as well as used for Ox and Cows to improve their health in snowy season (Shoaib *et al*., 2021).

1. MATERIALS AND METHODS

2.1Plant Collection:

Whole plant of *Isodon rugosus* was collected from Patrak District Dir Upper Khyber Pakhtunkhwa (**Lat:** 35.35781 N35° 21'28.122''; **Long:** 72.0641 E72° 3'23.07'') and *Phytolacca latbenia* was collected from Arangkel Azad Kashmir (**Lat:** 34.808667° N34°48'31''; **Long:** 74.35031 E74° 21'1''during September 2022. Arial parts of *I. rugosus* and *P. latbenia* were washed and shade dried at room temperature. The dried plants were ground through Grinder to fine powder (1mm diameter).

2.2 Preparation of Plants Extract:

The ground powder of each sample was used for extract preparation. Ethanol and methanol were used as solvent for the preparation of plants extract. 500 gram of both plant powders (whole plant) were added to 95% ethanol and methanol at room temperature and keep for 72hours with proper steering after each 24houres and the same was repeated three times. Watman filter paper-1 was used to filter the extract and then filtrates were pooled together. The pooled filtrated was concentrated with rotary evaporator (Laborota 4000 Germany) at 40^0C under lower pressure . Final dried and concentrated extract was weighted which was stored in refrigerator for further processing different biological activities.

3.3 Phytochemical Qualitative Analysis

Phytochemical qualitative activity of *I. rugosus* and *P. latbenia* was carried out in the laboratory of Qurtuba University of Science and Information Technology Peshawar (QUSIT) after plant extract preparation. The following detailed protocols were used for qualitative assay:

3.3.1 Test for Saponins

Frothing test: The plant extract (5 ml) was taken in a test tube and agitate it by shaking. The froth formation specified the existence of saponins (Chaouche *et al.,* 2011).

3.3.2 Test for Steroids

Salkowski's test: 2ml of chloroform and concentrated sulfuric acid (H_2SO_4) was added to 5ml of the extract. The indication of a red color in the lower chloroform layer showed the occurrence of steroids (Harborne, 1998).

3.3.3 Test for Glycosides Salkowski's test

Mixed 3ml of the extract solution with 2ml of chloroform and then carefully added concentrated Sulfuric acid (H_2SO_4) about 2ml and the mixture was gently shaken. The formation of a radish-brown color indicated the existence of a steroidal ring (Harborne, 1998).

3.3.4 Test for Terpenoids Salkwski's test

To the extract solution few drops of the chloroform and sulfuric acid were added. The appearance of grayish colour designated the occurrence of terpenoids (Harborne, 1998).

3.3.4 Test for Flavonoids

The extract was treated with sodium hydroxide solution .The detection of flavonoids is indicated by the formation of a yellow red-precipitation (Kokate, 2008).

3.3.6 Test for Phenols and tannins Ferric chloride test

Added 2ml of ferric chloride to 2ml of extract solution. The existence of phenol was confirmed by the emergence of a deep bluish-green color. When the extract solution was combined with $FeCL_3$, the occurrence of a blue green colour showed the occurrence of tannins (Dahiru *et al.,* 2006).

3.3.7 Total Phenolic Assay Folin Ciocalteu test

The quantification of total phenols was investigated using Folin-Ciocalteu assay as described by (Oliveira *et al*., 2008). The plants samples extract about 0.1g was mixed with 60-80ml of distilled water, heated and boiled for about 30min in a sand bath. The resulting mixture was then filtered and the final volume was adjusted to 100ml with distilled water. In subsequent step 0.1ml of the extract was added to the test tube (Screw cap) along with 3.9ml distilled water and the mixture was agitated. Further 1ml of Folin-Ciocalteu reagent and 5ml of 20% sodium carbonate were added and the solution was dynamically shaken. The mixtures were kept at a room temperature for 20min. The optical density (O.D) of the sample was measured at a wavelength of 720nm. All experiment was conducted in triplicate.

3.3.8. Test for Carbohydrates Detection Fehling's Test

Sample solution was taken in the test tube and added some drops of equal volume of Fehling's A regent (Copper sulfate in distilled water) followed by Fehling's B regents (Potassium tartarate and hydroxide in distilled water). The mixture was boiled. The appearance of a brick-red colour in the precipitation of cuprous oxide determined the existence carbohydrates (Evan, 2002).

3.3.9. Test for Protein Detection Ninhydrin Test:

The extract solution in a test tube was treated with a 0.2% Ninhydrin solution. The presence of volatile coloration indicated the presence of protein (Kumar & Kiladi, 2009).

3.3.10. Test for Alkaloid Detection Wagner's Test

To the sample solution added some drops of Wagner's regent. The alkaloids detection was indicated due to the presence of red precipitation in the mixture (Khandelwal, 2004).

3.3.11. Test for Phytosterols and Terpenoids Salkowski's test

To the extract solution add little amount of sulfuric acid and chloroform. The appearance of yellow colour point out the presence of phytosterols whereas the appearance of red-colour at the lower side showed tri-terpenoids (Harborn, 1998).

3.3.12. Test for Anthocyanin Hydrochloric acid Test

Added 2ml of HCL solution with 2ml of extract solution. The appearance of bluish-green colour showed the existence of anthocyanin (Harborn, 1998).

3.3.13. Test for Phenolic Compounds Ferric chloride Test

Added 2ml of ferric chloride with 2ml of extract solution. The determination of phenol was confirmed by the appearance of bluish-green colour (Dahiru *et al.,* 2006).

3.4. Phytochemical Quantitative Tests

Different quantitative tests of ethanolic and methanolic extract of *I. rugosus* and *P. latbenia* were performed in the laboratory of Qurtuba University of Science and Information Technology Peshawar (QUSIT) to determine alkaloids, phenol, tannins and flavonoids quantitatively.

3.4.1 Determination of Alkaloids

The determination of alkaloids was conducted through the alkaline precipitation gravimetric method outlined by (Harborn, 1973). With each of the ethanolic and methanolic extract (5gram each), 50ml of 10% acetic acid solution were the resulting mixture was shaken and left for 4 hours in stationary position. Whatman No-42 filter paper

was used to filter the mixture. The filter obtained was then to one quarter of its original volume through evaporation on a steam bath. Alkaloids in the extract were precipitated by slowly adding ammonium hydroxide (NH_4OH) until complete turbidity was achieved. The precipitated alkaloids were recovered through filtration using a pre-weighed filter paper, followed by washing with 1% ammonium solution (NH_4OH). The collected precipitation was then dried in an oven at 80°C for 60 minutes, cooled in a desiccator and weighted again. The weight difference before and after the precipitation process determined the weight of alkaloids, expressed as percentage of the initially analyzed sample. The calculation was performed using the formula:

$$\%\text{alkaloids} = \frac{W2 - W1}{W1} \times 100$$

Where

W = weight of sample. W1 = weight of empty filter paper.

W2 = weight of paper + alkaloid precipitate

3.4.2 Determination of Phenols

The quantification of phenols was conducted through the Folin-Ciocalteu colorimetric method as outlined by (Pearson 1976). To initiate the process, 0.2g of sample was introduced into a test tube, followed by the addition of 10ml of ethanolic and methanolic plants extract and shaking. Subsequently, 1ml of the resulting extract was transferred to another test tube and 1ml of Folin-Ciocalteu reagent, diluted with 5ml of distilled water was added. The absorbance of the developed color was then measured at a wavelength of 760nm.

3.4.3 Determination of Tannins:

The determination of tannins was performed by the methods described by (Peri and Pompei 1971). The ethanol and methanol were used as solvents. 1ml of both ethanolic and methanolic extracts with a concentration of 1mg/ml was placed into separate test tubes. The volume was adjusted to 1ml using distilled water while 1ml of water serves as the blank. To each test tube 0.5nl of Folin's phenol regents (diluted at a ratio of 1:2) was added followed by addition of 5ml of 35% sodium carbonate solution. The mixture was then kept at room temperature for 5min. The formation of blue color was observed and the color of the color was measured at 640nm using spectrometer. A standard graph using gallic acid at a concentration of 1ml was plotted to determine the tannins contents of the extract. The total tannins contents was expressed in mg/g of the extract

3.4.4 Determination of Flavonoids

Determination of flavonoids was assessed using the methods outlined by (Harborne 1973). 5g of both ethanolic and methanolic extracts were subjected to boiling in 100ml of a 2M hydrogen chloride solution for duration of 30minutes. After boiling the mixture was allowed to cool and subsequently filtered through Whatman No. 42 filter paper. The resulting filtrate was treated with ethyl acetate added drop-wise until it was in excess. The precipitated flavonoids were then collected through filtration using a weighted filter paper. The collected materials were dried in an oven at 80°C, cooled a desiccator and re-weighed. The variance in weight provided the quantity of flavonoids, expressed as a percentage of the initially analyzed sample weight. The percentage of the flavonoids was determined using the formula.

$$\%\text{Flavonoids} = \frac{W2 - W2}{W1} \times 100$$

Where:

W = weight of the sample analyzed, W1 = Weight of the empty crucible

W2 = Weight of the filter paper + flavonoids precipitate.

3.10. Analgesic Activity

Hot Plate Method

Requirements

BALB/C mice having weight about 22 gram, Diclofenac sodium, normal saline (used as control) and plants extract of (*I. rugosus* and *P. latbenia*).

Procedure

Healthy BALB/C mice having weight (20g) were collected from Veterinary Research Institute (VRI) Peshawar and were kept on fast for 24 hours before starting experiment. The BALB/C mice of either sex were randomly assigned to five groups each comprising of five mice. The first group was treated with normal saline (10ml/kg) and designated as control group. The second group was treated with standard drug (Diclofenac sodium) at a dose of 10mg/kg. Groups 3, 4 and 5 were orally given various concentration (100, 200, and 300mg/kg, body weight) of ethanolic and methanolic extract of plants. To assess the initial reaction time the mice were placed on a hot plat set at 55± 0.5°C. The index of reaction to heat was determined by observing paw licking or jumping. Post treatment of the reaction time of each animal was recorded at 30, 60 and 90 minutes after the administration of the plants extracts. To assess the activity followed the protocol and methods out lined by (Eddy & Leimbach ,1953). Percent elongation was calculated by;

$$\text{Elongation (\%)} = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (test)}} \times 100$$

Statistical analysis
To analyze the data obtained from analgesic activity the dunnet's t-test was applied. For the statistical tests p-values of less than 0.001, 0.01 and 0.05 were recorded as significant.

3.11. Antispasmodic Activity
Requirements
BALB/C mice, plants extract (*I. rugosus* and *P. latbenia*) and charcoal.

Procedure
Healthy BALB/C mice with a weight of 20g were obtained from the Veterinary Research Institute (VRI) in Peshawar. These mice were maintained under standard environmental condition receiving a standard diet and water. The experiment was performed after an overnight fast. The mice were divided into three groups; control, positive control and test group, each consisting of 5 mice. All groups were administered 1ml of charcoal meal orally. The test groups were given ethanolic and methanolic extract of plants at dose of 100, 200 and 300mg/kg. the positive control group received castor oil (10ml/kg) while the control group received distilled water(10ml/kg). After 50 minutes of charcoal treatment each mouse was killed and the distance travelled by charcoal meal from pylorus to caecum was measured. The peristaltic index represents the distance travelled by the charcoal meal to the total length of small intestine expressed in percentage (Yasmeen *et al*., 2010). The percent inhibition produced by extracts was calculated by formula

$$\%\text{Inhibition} = 100 - \frac{\text{PI (Control)-PI (Test)}}{\text{PI (control)}} \times 100$$

3.12. Anti-inflammatory Activity
The anti-inflammatory effect of ethanolic and methanolic extract of *I. rugosus* and *P. latbenia* were evaluated using the carrageenan-induced inflammation model in rate paw following the protocol described by (Winter & Poster 1957). Mice were divided into five groups, each group consist of five mice. Acute inflammation was induced in all groups by injecting 0.1ml of a freshly prepared 1% carrageenan suspension. Paw volume was measured using plethysommetry from 0-180 minutes after carrageenan injection. To pre-medicate the mice of all groups were orally administered diclofenac sodium at dose of (10mg/kg body weight) two hours before inducing inflammation. The mean increase in paw volume was measured and the percent increase was calculated all extract. The percent inhibition of paw volume was determined using the following formula;

$$\%\text{ Inhibition of paw edema} = \frac{Vc - Vt}{Vc} \times 100$$

Where
Vt= increase in paw volume in rate treated with test compounds
Vc = increase in paw volume in control group of rats.

Statistical Analysis
To analyze the data obtained from anti-inflammatory activity the dunnet's t-test was applied. For the statistical tests p-values of less than 0.001, 0.01 and 0.05 were recorded as significant.

3. Result and Discussion
3.1. Phytochemical Analysis *Isodon rugosus* (Wall. ex. Benth.) Codd
Both qualitative and quantitative phytochemical analysis of *Isodon rugosus* ethanolic and methanolic extracts were performed (Table. 3.1a and 3.1b).
Qualitative phytochemical analysis of ethanolic extract of *I. rugosus* indicated the presence of 11 phytochemical such as saponins, steroids, terpenoids, flavonoids, phenol, total phenolic, carbohydrate, protein, alkaloids, Anthocyanin, and phenolic compounds. While glycoside and tannins and phytosterol were found absent. These results are in line with Sunil *et al.* (2023) who also reported similar phytochemicals in the ethanolic extract of *Abutilon indicum*. The quantitative phytochemical analysis of *I. rugosus* revealed the existence of Alkaloids (49.19%), Phenols (37.30%), Tannins (51.39%) and Flavonoids (46.07%). The result is similar with Daniel *et al.* (2024) who also reported same phytochemicals quantitatively in *Adansonia digitata*. The result for the methanolic extract of *I. rugosus* showed the existence of 10 phytochemicals such as steroids, terpenoids, flavonoids, phenol, total phenolic, carbohydrate, protein, alkaloids and phenolic compounds while glycoside, tannins, phytosterol and anthocyanin were found absent. The same result of qualitative phytochemical screening was reported by Packirisamy *et al.* (2023) in the methanolic extract of *Picrorhiza kurroa*. On other hand the quantitative analysis of the methanolic extract of *I. rugosus* point out the presence of alkaloids (53.87%), Phenol (66.21%), Tannin (79.41%), flavonoids (73.53%). These results show similarity with Olalekan (2023) who reported similar quantitative phytochemicals in the *Bridella ferruginea* and *Piliostigma thinningii.*

Table 3.1a Qualitative Phytochemicals founds in the Ethanolic and Methanolic Extract of *Isodon rugosus* (Wall. ex. Benth.) Codd

S. No	Phytochemicals	Test	Observations	Ethanolic extract	Methanolic extract
1	Saponins	Frothing test	Forth formation	+	+
2	Steroids	Salkowski's test	Red color production	+	+
3	Glycoside	Salkowski's test	Reddish brown color	_	_
4	Terpenoids	Salkowski's test	Grayish colour appearance	+	+
5	Flavonoids	Alkaline reagent test	Yellow red precipitation	+	+
6	Phenol	Ferric chloride test	Deep bluish green color	+	+
7	Tannins	Ferric chloride test	Blue green color appearance	_	_
8	Total Phenolic	Folin Ciocalteu test	blue, green, red or purple color appearance	+	+
9	Carbohydrate	Fehling's test	Red color precipitation	+	+
10	Protein	Ninhydrine test	Violet coloration	+	+
11	Alkaloid	Wanger's test	Red precipitation	+	+
12	Phytosterole	Salkowski's test	Red color/ yellow color	_	_
13	Anthocyanin	Hydrochloric acid test	Bluish green color appearance	+	_
14	Phenolic compounds	Ferric chloride test	Bluish green color appearance	+	+

Table. 3.1b. Quantitative Phytochemicals found in Ethanolic and Methanolic Extract of *Isodon rugosus* (Wall. ex. Benth.) Codd

S. No	Extract	Alkaloids(mg/g)	Phenols (mg/g)	Tannins(mg/g)	Flavonoids(mg/g)
1	**Ethanol**	49.18	37.30	51.39	46.07
2	**Methanolic**	53.87	66.21	79.41	73.53

3.2. Analgesic Activity of Ethanolic and Methanolic Extracts of *Isodon rugosus* (Wall. ex. Benth) Codd

An analgesic is a substance within the category of drug utilized to achieve analgesia, which is the alleviation of pain without inducing loss of consciousness. Pain is a widespread and debilitating health issue treated through the use of medicinal (Christy, 2023). Pain is a distressing emotional event resulting from actual or potential tissue injury managed with various analgesic medications that can lead to sleepiness and drowsiness (Naeem *et al*., 2023). The ethanolic extract of *I rugosus* were carried out to investigate the analgesic potential. The result showed that the mean reaction time was recorded at different time intervals (0min, 30min, 60mim and 90min) along with the standard error of mean (SEM) and the percent decrease in latency time. The result revealed that highly increase in the latency time after 30min were noted (6.80±1.92), (9.20±0.92) and (9.40±0.14) at dose 100mg/kg, 200mg/kg and 300mg/kg individually, whereas increase in the latency time after 60min were recorded (6.50±0.68) at 100mg/kg fallowed by (7.40±0.56), (8.30±0.34) at dose 200mg/kg and 300 mg/kg. Similarly after 90min latency time (8.29±0.78) were recorded at dose 100mg/kg while at dose 200mg/kg and 300mg/kg the increase in latency time were noted (9.53±0.73) and (9.69±0.53) respectively. The maximum percent increase 27.45%, 26.23%, 15.19% in latency time of ethanolic extract was observed after 90mins of drug administration which was lower than diclofenac sodium. The diclofenac sodium showed high level of increase 32.46% in latency time (Table no 3.2). The similar results were obtained by Bhattacharya *et al*. (2014) while studying the analgesic effect of ethanolic extract of the leaves of *Moringa oleifera.* The result for methanolic extract of *I. rugosus* indicated a significant increase in latency time (9.12±0.90) at a dose of 300mg/kg while at dose of 100mg/kg and 200mg/kg latency time (7.00±0.85) and (8.60±0.75) were observed respectively, after 30min. After 60min the highest latency time (9.94±0.61) was noted at a dose of 300mg/kg followed by (9.53±0.65) and (8.76±0.81) at dose 200mg/kg and 100mg/kg. Similarly after 90min a substantial increase in latency time (10.04±0.83) was observed at a dose 300mg/kg while at dose 200mg/kg and 100mg/kg latency time were (9.23±0.30) and (9.19±0.47) respectively. The maximum percent increase 24.30%, 17.65%, 17.30% in latency time of methanolic extract was observed after 90mins of drug administration while lower than diclofanic sodium which revealed high level of increases 32.46% in latency time (Table no 3.2). The results obtained show similarity with Ayanaw (2023) who evaluated methanolic extract of *Gomphocarpus purpurascens* for their analgesic potential.

Table no 3.2. Analgesic activity of ethanolic and methanolic extract of *Isodon rugosus*

Treatment	Dose (mg/kg)	Mean reaction time by hot plate methods (Mean+SEM)				Percent Decrease in Latency time
		0min	30min	60min	90min	
Normal Saline	10ml/kg	6.48±0.43	7.40 ±0.17	6.90 ±0.41	7.03±0.48	
Diclofenac sodium	10 mg/kg	7.21±0.14	9.70 ±0.84	9.94±0.36±	10.41±0.23	32.46
Ethanolic extract	100mg/kg	5.47±0.30	6.80±1.92	6.50±0.68	8.29±0.78	15.19
	200mg/kg	5.91±0.33	9.20±0.92	7.40±0.56	9.53±0.73	26.23
	300mg/kg	6.09±0.81	9.40±0.14	8.30±0.34	9.69±0.53	27.45
Methanolic extract	100mg/kg	6.69±0.79	7.00±0.85	8.76±0.81	9.19±0.47	17.30
	200mg/kg	6.81±0.92	8.60±0.75	9.53±0.65	9.23±0.30	17.65
	300mg/kg	7.13±0.71	9.12±0.90	9.94±0.61	10.04±0.83	24.30

Key: *P<0.05, **P<0.01, ***P<0.001

3.3. Anti-spasmodic activity of *Isodon rugosus* (Wall. ex. Benth) Codd

Gastrointestinal issues are connected to disruption in motility, heightened visceral sensitivity and change in mucosal and immune function as well as weakened regulation through central nervous system (CNS) (Drossman, 2016). Antispasmodic synthetic drugs may cause various side effects such as weakness, and mouth dryness. Therefore, the determination of new molecules having anti-spasmodic potential is a vital aim for the pharmaceutical industry (Ybañez-Julca *et al*., 2023). The ethanolic and methanolic extract of *I. rugosus* was evaluated to find out the antispasmodic potential. The result exhibited that the ethanolic extract showed significant antispasmodic potential at doses of 100 and 200mg/kg while maximum result (15.28±1.17) was exhibited by 300mg/kg. The percent inhibition of charcoal's movement of ethanolic extract were 59.17%, 60.89%, 68.34% at doses of 100, 200 and 300mg/kg, whereas the percent inhibition of standard drug (Atropine sulphate) at a dose of 10mg/kg was (75.44%). Similarly the result for methanolic extract of I. rugosus showed significant antispasmodic activity (20.34±0.94) and (19.15±1.01) at a doses of 100 and 200mg/kg fallowed by minimum antispasmodic activity (16.80±1.07) at a dose of 300mg/kg. The percent inhibition of charcoal's movement for methanolic extract was observed 54.17%, 61.90%, and 65.00%, whereas the percent inhibition of standard drug (Atropine sulphate) at a dose of 10 mg/kg was observed 75.44%. The maximum reduction of charcoal movement was caused by mean of standard drug (atropine sulphate), whereas the maximum reduction of ethanolic and methanolic extract were observed at doses of 200 and 300mg/kg (Table 3.3). The plant extracts of both ethanolic and methanolic exhibit dose dependent manner inhibition of charcoal movement. The result obtained show similarity with (Islam *et al.,* 2013) who reported that the methanolic extract possessed similar inhibition of castor oil induced diarrhea. The antispasmodic potential of *I. rugosus* might be due the presence of phytochemicals like terpenoids, flavonoids, alkaloids and saponins which were determined in the plants extracts (Table 3.3). The results indicates resemblances with (Nitiema *et al*., 2023) who reported phytochemicals such as flavonoids, tannins, sterols, tri-terpenes and saponosides in *Diospyros mespiliformis* leaves and concluded that these are used in the treatment of diarrhea.

Table no 3.3 Antispasmodic activity of ethanolic and methanolic extract of *Isodon rugosus* (Wall. ex. Benth) Codd

S. No	Treatment	Dose (mg/kg)	Mean length of intestine	Mean distance travelled by charcoal	Percent inhibition %
1	Normal saline	10 ml/kg	46.37	38.31±2.2	17.38
2	Atropine sulphate	10mg/kg	47.32	11.62±.1.14	75.44
3	Ethanolic extract	100mg/kg	48.95	19.32±0.91	59.17
		200mg/kg	49.32	19.14±1.01	60.89
		300mg/kg	48.27	15.28±1.17	68.34
3	Methanolic extract	100mg/kg	44.39	20.34±0.94	54.17
		200mg/kg	50.27	19.15±1.01	61.90
		300mg/kg	48.00	16.80±1.07	65.00

Key; *P<0.05, **P<o.o1, ***P<0.001

3.4. Anti-inflammatory Activity of *Isodon rugosus* (Wall. ex. Benth) Codd

Inflammation is recognized as a crucial physiological response associated with the development of various chronic conditions including autoimmune disorder like diabetes, cancer, and arthritis (Chen *et al*., 2018; Furman *et al*., 2019). Basically inflammatory diseases are commonly cured through steroidal and non-steroidal anti-inflammatory drugs but their prolonged usage leads to various side effects (Pereira *et al*., 2023). Science ancient time people have relied on the natural compounds for cure of inflammation because usage of medicinal plants is considered safe, cost-effective and extensively accepted (Radovanović *et al*., 2023). The ethanolic and methanolic extracts of *I. rugosus* were evaluated for their anti-inflammatory potential (Table no 3.4). Result indicated that ethanolic extract of *I. rugosus* exhibit statistically significant inhibition in paw volume after drug administration (1.78±0.08) at dose 100mg/kg followed by (1.68±0.13) and (1.58±0.14) at a doses of 200 and 300mg/kg after 30minutes. While the highest paw volume after drug administration after 60minutes at doses of 100, 200 and 300mg/kg were noted (1.62±0.08), (1.52±0.08) and (1.48±0.08). Similarly high paw volume after drug administration after 90minutes at dose of 100mg/kg were observed (1.50±0.18) moderate paw volume at 200mg/kg were noted (1.48±0.13) and lowest paw volume (1.44±0.16) were recorded at 300mg/kg (Table no 3.4). The results are in line with Gadamsetty *et al*. (2013) who assessed anti-inflammatory potential of ethanolic extract of *Mimusops elengi* leaves. The result for methanolic extract of *I. rugosus* revealed the highest paw volume after drug administration (1.78±0.08) at a dose of 100mg/kg, followed by (1.68±0.13) on both 200 and 300mg/kg doses after 30minutes. Similarly the highest paw volume after drug administration after 60minutes and 90minutes were observed (1.58±0.08) and (1.66±0.11) respectively, while lowest paw volume after drug administration were recorded (1.46±0.11) and (1.54±0.11) respectively (Table 3.4). The results obtained are alike with Mayouf *et al.* (2019) who reported anti-inflammatory effect of methanolic extract of *Asphodelus microcarpus.* The percent inhibition in paw volume of ethanolic extract was 31.81%, 32.72% and 34.54% and that of methanolic extract was 24.54%, 27.27% and 30.00% respectively at doses of 100, 200 and 300mg/kg body weight of mice individually when matched with standard drug (Diclofenac sodium) at a dose of 10mg/kg which exhibit 47.27% decrease in paw volume (Table no 3.4). The ant-inflammatory potential of ethanolic and methanolic extracts of *I. rugosus* was both time and dose dependent while the ethanolic extract exhibit significant result in per cent decrease in paw volume but less than standard drug diclofanic sodium (Table 3.4). The anti-inflammatory potential of *I. rugosus* may be due to the existence of phytochemicals such as tannins, glycosides, glycosides and saponins which were indicated in both extracts of plant (Table 3.1a and 3.1b). Flavonoids also play an essential role in the biotransformation of prostaglandins (Gupta and Singh, 2017).

Table 3.4 Anti-inflammatory activity of ethanolic extract and methanolic extract of *Isodon rugosus* (Wall. ex. Benth) Codd

Treatment	Dose (mg/kg)	Paw Volume After Drug Administration (Mean + SEM)			Percent Decrease in Paw volume (%)
		30min	60min	90min	
Normal Saline	10mg/kh	1.82 ± 0.08	2.08±0.03	2.20±0.05	0.00
Diclofenac sodium	10mg/kg	1.72±0.02	1.34±0.11	1.16±0.04	47.27
Ethanolic extract	100mg/kg	1.78±0.08	1.62±0.08	1.50±0.18	31.81
	200mg/kg	1.68±0.13	1.52±0.08	1.48±0.13	32.72
	300mg/kg	1.58±0.14	1.48±0.08	1.44±0.16	34.54
Methanolic extract	100mg/kg	1.78±0.08	1.58±0.08	1.66±0.11	24.54
	200mg/kg	1.68±0.14	1.51±0.08	1.60±0.09	27.27
	300mg/kg	1.68±0.13	1.46±0.11	1.54±0.11	30.00

Key: *P<0.05, **P<0.01, ***P<0.001

3.5. Phytochemical analysis *Phytolacca latbenia* (Moq.) H. Walter

Phytochemical screening reveal abstraction, screening and identification of phytochemical constituents present in various plants and are used in the manufacturing of useful drugs (Okoli *et al*., 2009). The qualitative and quantitative screening of phytochemicals aims to find out the highest groups of chemical compounds including saponins, alkaloids, carbohydrates, tannins, terpenoids, phenol and flavonoids of the extracts (Savithramma *et al*., 2011; Senguttuvan *et al*., 2014). Both

qualitative and quantitative phytochemical analyses of ethanolic and methanolic extracts of *Phytolacca latbenia* were performed (Table 3.5a and 3.5b). Qualitative phytochemical analysis of ethanolic extract of *P. latbenia* showed the presence of 11 phytochemicals such as steroids, terpenoids, flavonoids, phenol, total phenolic, carbohydrate, alkaloids, Phytosterole and Terpenoids, Anthocyanin and phenolic compounds while saponins, glycoside ,tannins and proteins. While the result for methanolic extract of *P. latbenia* exhibit occurrence of phytochemicals namely; saponins, steroids, terpenoids, flavonoids, carbohydrates, phenols, total phenolic, phytosterol and phenolic compounds (Table 3.5a). The results obtained shows similarity with the results reported by Hossen *et al*. (2023) who studied phytochemical analysis of *Dalbergia Sissoo* and reported same phytochemicals. Quantitative phytochemical analysis of methanolic extract of *P. latbenia* indicated the occurrence of alkaloid (81.36%), Phenol (78.12%), Tannins (45.09%), Flavonoids (53.19%) whereas the *P. latbenia* ethanolic extract exhibit the existence of alkaloids (73.70%), Phenol (59.86%), Tannin (34.27%) and Flavonoids (61.41%) **(**Table 3.5b).These findings are parallel with Madhu *et al*. (2016) they performed quantitative photochemical analysis of various medicinal plants through various organic solvent including ethanol and methanol.

Table No. 3.5a Qualitative Phytochemical Analysis of Both Ethanolic and Methanolic Extract of *Phytolacca latbenia* (Moq.) H. Walter

S. No	Phytochemicals	Test	Observations	Ethanolic extract	Methanolic extract
1	Saponins	Frothing test	Forth formation	_	+
2	Steroids	Salkowski's test	Red color production	+	+
3	Glycoside	Salkowski's test	Reddish brown color	_	_
4	Terpenoids	Salkowski's test	Grayish colour appearance	+	+
5	Flavonoids	Alkaline reagent test	Yellow red precipitation	+	+
6	Phenol	Ferric chloride test	Deep bluish green color	+	+
7	Tannins	Ferric chloride test	Blue green color appearance	_	_
8	Total Phenolic	Folin Ciocalteu test	Blue, green, red or purple color appearance	+	+
9	Carbohydrate	Fehling's test	Red color precipitation	+	+
10	Alkaloid	Wanger's test	Red precipitation	+	_
11	Protein	Ninhydrine test	Violet coloration	_	_
12	Phytosterole and Terpenoids	Salkowski's test	Red color/ yellow color	+	+
13	Anthocyanin	Hydrochloric acid test	Bluish green color appearance	+	_
14	Phenolic compounds	Ferric chloride test	Bluish green color appearance	+	+

Table No. 3.5b Quantitative phytochemicals found in ethanolic and methanolic extract of *Phytolacca latbenia* (Moq.) H. Walter.

S. No	Extract	Alkaloids (mg/g)	Phenols (mg/g)	Tannins (mg/g)	Flavonoids (mg/g)
1	**Methanol**	81.36	78.12	45.09	53.19
2	**Ethanol**	73.70	59.86	34.27	61.41

3.6 Analgesic Activity of Ethanolic Extract of *Phytolacca latbenia* (Moq.) H. Walter

There is a growing focus on studying natural products to identify compounds that can interact with established analgesic target (McCurdy & Scully, 2005). Some medicinal plants, such as Willow bark (*Salix spp*) and Turmeric (*Curcuma longa*), having analgesic properties, which can help to relieve pain (Ghutke *et al*., 2023). The ethanolic extract of *P. Latbenia* was evaluated for their analgesic potential. The mean reaction time is recorded at different time intervals (0min, 30min, 60mim and 90min) along with the standard error of mean (SEM) and the percentage decrease in latency time. The results indicated that a significant increase in latency time (6.90±0.39) occurred at a dose of 300mg/kg, followed by (6.34±0.44) at 200mg/kg and the lowest latency time (5.96±0.42) noted at a dose of 100mg/kg after 30min. After 60min the latency time at 300mg/kg was notably high (8.17±0.37), followed by doses of 200mg/kg and 100mg/kg which are (7.91±0.55) and

(7.02±0.67). whereas the latency time after 90min the highest value(10.09±0.16) were recorded at dose of 300mg/kg, with moderate latency time(9.33±0.43) at 200mg/kg and the lowest latency time (8.02±0.25) at doses of 200mg/kg. The ethanolic extract demonstrated a maximum percent increase of 48.36%, 44.15%, and 35.03% in latency time after 90minutes of drug administration. However these increase were lower than those observed with diclofenac sodium which exhibited a higher level increase of 54.69% in latency time. The results obtained are in-line with Idu *et al*. (2023) who studied analgesic potential of *Boswellia dalzielii*.

The result for methanolic extract of *P. latbenia* showed latency time (4.26±0.21), (5.60±0.51) and (6.22±0.67) at dose 100, 200 and 300mg/kg individually after 30minutes while latency time after 60mintutes at dose of 100, 200 and 300mg/kg were noted (5.60±0.51), (6.13±0.70) and (7.60±0.20). Similarly the significant latency time (9.49±0.14) were observed at 300mg/kg followed by (7.51±0.54) and (6.97±0.42) at dose 200 and 300mg/kg after 90minutes. The methanolic extract demonstrated a maximum percent increase of 51.94%, 39.28%, and 34.57% in latency time after 90minutes of drug administration. However these increase were lower than those observed with diclofenac sodium which exhibited a higher level increase of 54.69% in latency time (Table 3.6). The results are alike with Asefa *et al.* (2022) who investigated analgesic activity of methanolic extract of *Verbascum sinaiticum*.

Table no 3.6. Analgesic activity of ethanolic and methanolic extracts of *Phytolacca latbenia* (Moq.) H. Walter

Treatment	Dose (mg/kg)	Mean reaction time by hot plate methods (Mean+SEM)				Percent decrease in latency time
		0min	30min	60min	90min	
Normal saline	10ml/kg	4.32±0.43	4.68±0.36	5.09±0.76	5.21±0.99	**0.00**
Diclofenac sodium	10ml/kg	5.76±0.05	7.00±0.78	8.40±0.92	11.5±0.15	**54.69**
Ethanolic extract	100mg/kg	4.15±0.14	5.96±0.42	7.02±0.67	8.02±0.25	**35.03**
	200mg/kg	4.83±0.70	6.34±0.44	7.91±0.55	9.33±0.43	**44.15**
	300mg/kg	5.08±0.21	6.90±0.39	8.17±0.37	10.09±0.16	**48.36**
Methanolic extract	100mg/kg	3.59±0.26	4.26±0.21	5.60±0.51	6.97±0.42	**34.57**
	200mg/kg	4.19±0.23	5.60±0.51	6.13±0.70	7.51±0.54	**39.28**
	300mg/kg	4.41±0.20	6.22±0.67	7.60±0.20	9.49±0.14	**51.94**

Key: *P<0.05, **P<0.01,***P<0.001

3.7. Antispasmodic activity of ethanolic and methanolic extracts of *Phytolacca latbenia* (Moq.) H. Walter

Functional gastro-intestinal dysmotility represent a significant aspect of dietary issues in the human population. Among these disorders are abdominal spasm, abdominal pain, and issues leading to colic such as diarrhea syndrome. For the treatment of these ailments the human often turn to the use of medicinal plants (Ouahhoud *et al*., 2023). The ethanolic and methanolic extracts of *P. latbenia* were evaluated to find out the antispasmodic potential.

The result exhibited that the ethanolic extract of *P. latbenia* shows high significant antispasmodic potential at a doses of 100 and 200mg/kg while maximum result (25.71±4.37) was exhibited by 300mg/kg. the percent inhibition of charcoal movement in the ethanolic extract was 48.51%, 48.65%, 51.74% at doses of 100, 200 and 300mg/kg, Whereas the percent inhibition of Atropine sulphate (standard drug) at dose of (10 mg/kg) was observed 57.22%. The result for methanolic extract of P. latbenia showed significant antispasmodic activity (30.26±2.94) and (28.18±3.38) at doses of 100 and 200mg/kg fallowed by lowest antispasmodic activity (26.64±2.81) at 300mg/kg. The percent inhibition of charcoal movement for methanolic extract was 42.28%, 45.49%, and 47.60%, however the percent inhibition of standard drug (Atropine sulphate) at a dose of (10 mg/kg) was detected 57.22%. The significant reduction of charcoal movement was produced due to standard drug (atropine sulphate) followed by ethanolic as well as methanolic extracts at a doses of 200 mg/kg and 300 mg/kg (Table 3.7). Bothe methanolic and ethanolic extract of *P. latbenia* exhibits dose-dependent manner inhibition of charcoal movement.

The results obtained indicate resemblance with Chaulya *et al*. (2011) who also reported the same result for the methanolic extract of *Cyperus tegetum.* The antispasmodic potential of *P. latbenia* might be due to presence of the phytochemical compounds such as flavonoids, saponins, terpenoids, alkaloids and tannins indicated in the extract of *P. latbenia* (Table 3.5a and 3.5b). Flavonoids and steroids are effective in preventing

secretion triggered by coaster oil and promote absorption of electrolytes by discharging prostaglandin (Tadesse *et al.,* 2017). Alkaloids and terpenoids inhibit secretion induce by castor oil by blocking the release of autacoids and prostaglandin (Okwuosa *et al*., 2016).

Table no. 3.7 Antispasmodic potential of ethanolic and methanolic extract of *Phytolacca latbenia*(Moq.) H. Walter

S.NO	Treatment	Dose (mg/kg)	Mean length of intestine	Mean distance travelled by charcoal	Percent inhibition %
1	Normal saline	10 ml/kg	50.58	43.21±1.87	18.52
2	Atropine sulphate	10mg/kg	50.19	21.47±3.90	57.22
3	Ethanolic extract	100mg/kg	53.94	27.77±2.83	48.51
		200mg/kg	51.86	26.63±2.66	48.65
		300mg/kg	53.28	25.71±4.37	51.74
3	Methanolic extract	100mg/kg	52.43	30.26±2.94	42.28
		200mg/kg	51.70	28.18±3.38	45.49
		300mg/kg	50.84	26.64±2.81	47.60

3.8. Anti-inflammatory of ethanolic and methanolic extract of *Phytolacca latbenia* (Moq.) H. Walter

Plants are the main source of molecules to develop new drugs having high therapeutic potential especially relating to anti-inflammatory activities. Ant-inflammatory drugs have a vital role in pathophysiological process of inflammation, to reduce tissues injury and provide comfort to the patients (Nunes *et al*., 2020). So it is desirable to discover new therapeutic agents for resolving inflammation. The treatment of inflammation involves targeting specific mechanism (Liu *et al*., 2017). Medicinal plants contain secondary metabolites and these secondary metabolites possess diagnostic potential and paly key role in the discovery and production of effective (Li *et al*., 2020).

The ethanolic as well as methanolic extract of *P. latbenia* was evaluated for their anti-inflammatory potential. Result showed that the ethanolic extract of *P. latbenia* possess significant inhibition in paw volume after drug administration (1.82±0.08) followed by (1.80±0.07) and (1.74±0.11) at a doses of 100, 200 and 300mg/kg after 30minutes. While the highest paw volume after drug administration after 60minutes at dose of 100, 200 and 300mg/kg were noted (1.76±0.11), (1.68±0.14) and (1.54±0.11) respectively. Whereas high paw volume after drug administration after 90minutes at dose of 100mg/kg were observed (1.64±0.11) moderate paw volume at dose of 200mg/kg were noted (1.56±0.11) and lowest paw volume (1.44±0.11) were recorded at dose of 300mg/kg when compared to standard drug (diclofenac sodium). Similarly the result for methanolic extract of *P. latbenia* showed the highest paw volume after drug administration (1.72±0.08) at dose of 100mg/kg, followed by (1.70±0.10) at dose of 200mg/kg and (1.66±0.11) at dose of 300mg/kg after 30minutes. Similarly the highest paw volume after drug administration after 60minutes and 90minutes were observed (1.58±0.08) and (1.48± 0.08) respectively, while lowest paw volume after drug administration were recorded (1.54±0.11) and (1.20±0.04) respectively (Table 3.8).

The results show equality with Ivan *et al*. (2023) who also reported anti-inflammatory properties of the methanolic extract of *Sida cuneifolia*. The percent inhibition in paw volume of ethanolic extract of *P. latbenia* was (18.81%), (22.77%) and (28.71%) and that of methanolic extract was (26.73%), (28.71%) and (40.59%) at a doses of 100, 200 and 300mg/kg body weight of mice respectively. When compared with diclofenac sodium (Standard drug) at a dose of 10mg/kg which indicated (45.04%) decrease in paw volume (Table 3.8).

The anti-inflammatory potential ethanolic extract of *P. latbenia* was both time and dose dependent while the methanolic extract exhibit significant result in percent decrease in paw volume (40.59%) at dose of 300mg/kg but less than standard drug diclofanic sodium (Table 4.2.9). *P. latbenia* shows anti-inflammatory potential due to the occurrence of flavonoids, polyphenols and terpenes. The flavonoids (anthocyanidins and flavones), polyphenols and terpenes are bioactive constituents are essential for anti-inflammatory disorders (Oguntibeju, 2018).

3.8. Anti-inflammatory activity of ethanolic and methanolic extract of *Phytolacca latbenia* (Moq.) H. Walter

Treatment	Dose (mg/kg)	Paw Volume after Drug administration (Mean + SEM)			Percent Decrease in Paw volume (%)
		30min	60min	90min	
Normal Saline	10ml/kg	1.80±0.10	1.96±0.18	2.02±0.03	0.00
Diclofanic sodium	10mg/kg	1.32±0.08	1.16±0.04	1.11±0.05	45.04
Ethanolic extract	100mg/g	1.82±0.08	1.76±0.11	1.64±0.11	18.81
	200mg/g	1.80±0.07	1.68±0.14	1.56±0.11	22.77
	300mg/g	1.74±0.11	1.54±0.11	1.44±0.11	28.71
Methanolic extract	100mg/g	1.72±0.08	1.58±0.14	1.48± 0.08	26.73
	200mg/g	1.70±0.10	1.60±0.10	1.44±1.16	28.71
	300mg/g	1.66±0.11	1.54±0.11	1.20±0.04	40.59

Key: *P<0.05, **P<0.01, ***P<0.001

Conclusion

Phytochemical analysis of ethanolic and methanolic extract of *Isodon rugosus* Wall. ex. Benth and *Phytolacca latbenia* (Moq.) H. Walter showed various secondary metabolites and these metabolites are used in the treatment of many ailments. Therefore these plants may also be used for dealing many disorders. The analgesic activity of ethanolic and methanolic extract of *I. rugosus* showed dose-dependent analgesic potential. Similarly the ethanolic and methanolic extract of *P.* latbenia also showed dose-dependent analgesic potential. These plants composed of various novels chemical compounds that help in the treatment of analgesic effect. The antispasmodic activity of ethanolic and methanolic extracts of both *I. rugosus* and *P. latbenia* exhibited significant decrease in charcoal movement in the intestine of tested organisms, which determined the antispasmodic potential of these plants. So the crude drug isolated from these plants may be used against spasmodic disorders. The anti-inflammatory activity of ethanolic and methanolic extract of *I. rugosus* and *P. latbenia* showed dose dependent percent decrease in paw edema which supports the traditional use of these plants against inflammatory disease.

References

1. Adetunji, C. O., Egbuna, C., Oladosun, T. O., Akram, M., Micheal, O. S., Olisaka, F. N., ... & Olaniyan, O. T. (2021). Efficacy of Phytochemicals of Medicinal Plants for the Treatment of Human Echinococcosis: Echinococcal Disease, Hydatidosis, or Hydatid Disease Drug Discovery. *Neglected Tropical Diseases and Phytochemicals in Drug Discovery*, 225-243.**Doi.org/10.1002/9781119617143.ch8**
2. Akhlaq, M., Alum, M. K., & Alam, M. M. (2022). Anti-inflammatory potential of medicinal plants. *Mediterranean Journal of Pharmacy and Pharmaceutical Sciences*, *2*(1), 15-23.
3. Akinmurele, O. J., Sonibare, M. A., Elujoba, A. A., Ogunlakin, A. D., Yeye, O. E., Gyebi, G. A., ... & Alanzi, A. R. (2023). Antispasmodic Effect of *Alstonia boonei* De Wild. and Its Constituents: Ex Vivo and In Silico Approaches. *Molecules*, *28*(20), 7069. **DOI.org/10.3390/molecules28207069**
4. Asefa, M., Teshome, N., & Degu, A. (2022). Anti-inflammatory and analgesic activity of methanolic root extract of *Verbascum sinaiticum* benth. *Journal of Inflammation Research*, 6381-6392.
5. Ayanaw, M. A., Yesuf, J. S. & Birru, E. M. (2023). Evaluation of Analgesic and Anti-inflammatory Activities of Methanolic Leaf and Root Extracts of *Gomphocarpus purpurascens* A. Rich (Asclepiadaceae) in Mice. *Journal of Experimental Pharmacology*, 1-11.
6. Bhattacharya, A., Agrawal, D., Sahu, P. K., Kumar, S., Mishra, S. S., & Patnaik, S. (2014). Analgesic effect of ethanolic leaf extract of *Moringa oleifera* on albino mice. *Indian journal of Pain*, *28*(2), 89-94.
7. Chaouche T, Haddouchi F, Bekkara FA (2011). Phytochemical study of root and leaves of the plant *Echium pycmanthum* Pomel. Der Pharm. Lett. 3(2):1-4
8. Chaulya, N.C., P.K. Haldar and A. Mukherjee. (2011). Antidiarrhoeal activity of methanol extract of the rhizomes of *Cyperus tegetum* Roxb. *Int. J. Pharm. Pharm. Sci*., 3(1): 133-135.
9. Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204
10. Christy, A. O. (2023). In Vivo Evaluation of Analgesic Activities of *Dioscorea dumetorum* (Kunth) Pax and *Tragia benthamii* Bak. *Sch Int J Tradit Complement Med*, *6*(2), 18-22.
11. Dahiru, D., Sini, J. M., & John-Africa, L. (2006). Antidiarrhoeal activity of *Ziziphus mauritiana*

root extract in rodents. *African journal of biotechnology*, *5*(10).

12. Dahiru, D., Sini, J. M., & John-Africa, L. (2006). Antidiarrhoeal activity of Ziziphus mauritiana root extract in rodents. *African journal of biotechnology*, *5*(10).
13. Daniel, J. L., Vandi, P., Chessed, G., Umar, M., & Yusuf, A. I. (2024). Phytochemical Analysis of Medicinal Plants Used in the Treatment of Malaria Infection in Billiri and Funakaye Local Government Areas of Gombe State. *BIMA JOURNAL OF SCIENCE AND TECHNOLOGY (2536-6041)*, *8*(1B), 287-298.
14. Drossman, D. A. (2016). History of functional gastrointestinal symptoms and disorders and chronicle of the Rome Foundation. *Rome IV functional gastrointestinal disorders: disorders of gut-brain interaction. Raleigh: Rome Foundation, Inc*, 549-576.
15. Eddy, N.B. and D. Leimbach. 1953. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J. Pharma. Exp. Therap*., 107(3): 385–393.
16. Evans WC (2002). Pharmacognosy, 15th ed. English Language Book, Society Baillere Tindall: Oxford University Press.
17. Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., ... & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature medicine*, *25*(12), 1822-1832.
18. Gadamsetty, G., Maru, S., & Sarada, N. C. (2013). Antioxidant and Anti-inflammatory Activities of the Methanolic Leaf Extract of Traditionally Used Medicinal Plant *Mimusops elengi* L. *Journal of pharmaceutical sciences and research*, *5*(6), 125.
19. Ghutke, T. D., Parvin, K., Rashida Banu, A. M., Bansal, S., Srivastava, A., Rout, S., & Ramzan, U. (2023). A comprehensive review on the therapeutic properties of medicinal plants. *Acta Traditional Medicine. V2i01*, 13-00.
20. Gupta, S. and A. Singh. 2017. Antimicrobial, analgesic and anti-inflammatory activity reported on *Tamarindus indica* Linn root extract. *Pharmacog. J.*, 9(3): 410-416.
21. Harborne, J.B. (1998) Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition, Chapman and Hall Ltd, London, 21-72.
22. Harborne, J.B. 1973. Photochemical methods: A guide to modern techniques of plant analysis.Chapman A. & Hall. London, pp: 22(9): 279.
23. Hossen, M. F., Nijhu, R. S., & Khatun, A. (2023). A phytochemical and pharmacological review on *Dalbergia sissoo*: A potential medicinal plant. *Journal of Pharmacognosy and Phytochemistry*, *12*(1), 52-57.
24. Idu, M., Omoregbee, A., & Gabriel, B. O. (2023). Phytochemical, Antioxidant Screening, Antinociceptive, and Anti-inflammatory Activities of *Boswellia dalzielii* Hutch (Burseraceae) Root Ethanol Extract Using Animal Model. *Biology, Medicine, & Natural Product Chemistry*, *12*(1), 143-150.
25. Islam, M. M., Pia, R. S., Sifath-E-Jahan, K., Chowdhury, J., Akter, F., Parvin, N., & Akter, S. (2013). Antidiarrheal activity of *Dillenia indica* bark extract. *International Journal of Pharmaceutical Sciences and Research*, *4*(2), 682.
26. Ivan, K., Abdelgadir, A. A., & Crispin, S. D. (2023). Comparative Evaluation of the Anti-Inflammatory Properties of Methanol and Aqueous Crude Extracts of Apical leaves of Sida cuneifolia: An Ethnomedicinal Plant. *Journal of Complementary and Alternative Medical Research*, *24*(4), 16-26.
27. Janbaz K.H., J. Arif, F. Saqib, I. Imran, M. Ashra, M. Zia-Ul-Haq, H.Z. Jaafar and V. De Feo (2014). In vitro and in-vivo validation of ethno pharmacological uses of methanol extract of *Isodon rugosus* Wall. ex Benth.(Lamiaceae). BMC Complement. Altern. Med 14(1): 71-82.
28. Khandelwal KR (2004). Practical Pharmacognosy, Techniques and experiments (12th Edn). Nirali Prakashan, Pune India. P 157.
29. Kokate, A., Li, X., & Jasti, B. (2008). HPLC detection of marker compounds during buccal permeation enhancement studies. *Journal of pharmaceutical and biomedical analysis*, *47*(1), 190-194.
30. Kumar P, B. J. R., & Kiladi S, C. P. Preliminary Phytochemical and Pharmacognostic Studies of *Holoptelea integrifolia* Roxb. *Ethnobotanical Leaflets*, *2009* (10), 3.
31. Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The scientific world journal*, *2013*(1), 162750.**Doi.org/10.1155/2013/162750**
32. Li, Y., Kong, D., Fu, Y., Sussman, M. R., & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, *148*, 80-89.
33. Liu, K. (2019). Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass. *Algal Research*, *40*, 101486.
34. Madaan, R., & Kumar, S. (2012). Screening of alkaloidal fraction of Conium maculatum L. aerial parts for analgesic and antiinflammatory activity. *Indian journal of pharmaceutical*

sciences, *74*(5), 457.DOI: 10.4103/0250-474X.108423

35. Madhu, M., Sailaja, V., Satyadev, T. N. V. S. S., & Satyanarayana, M. V. (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *Journal of pharmacognosy and phytochemistry*, *5*(2), 25-29.
36. Mayouf, N., Charef, N., Saoudi, S., Baghiani, A., Khennouf, S., & Arrar, L. (2019). Antioxidant and anti-inflammatory effect of Asphodelus microcarpus methanolic extracts. *Journal of ethnopharmacology*, *239*, 111914.
37. McCurdy, C. R., & Scully, S. S. (2005). Analgesic substances derived from natural products (natureceuticals). *Life Sciences*, *78*(5), 476-484.
38. Mendoza, N., & Silva, E. M. E. (2018). Introduction to phytochemicals: secondary metabolites from plants with active principles for pharmacological importance. *Phytochemicals: Source of antioxidants and role in disease prevention*, *25*, 1-5. DOI: 10.4103/0250-474X.108423
39. Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., & Waghorn, G. C. (2019). Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. *Crop Science*, *59*(3), 861-885. DOI: **org/10.2135/cropsci2017.06.0369**
40. Naeem, H., Gul, S., Khan, M., Hamid, S., Leghari, Q. A., Yasin, H., & Perveen, R. (2023). Anti-inflammatory and Analgesic Activity of *Cleome brachycarpa* Ethanolic Extract in Lower Mammals and Effects on Blood and Liver Enzymes. *Jundishapur Journal of Natural Pharmaceutical Products*.
41. Nawaz, H., Aslam, M., Shahzad, H., Mehboob, F., Waheed, R., Jabeen, R., ... & Ahmed, M. Z. (2022). Comparative Evaluation of Phytochemical Composition and Antioxidant potential of some Medicinally-important euphorbiaceous plants. *japs: Journal of Animal & Plant Sciences*, *32*(6). DOI: org/10.36899/JAPS.2022.6.0578
42. Nitiema, M., Ouedraogo, W. R. C., Rainatou, B. O. L. Y., Ouedraogo, P. E., Noura, O. M., Kaboré, B., ... & Ouédraogo, S. (2023). Phytochemical profile, acute oral toxicity, antioxidant, and antispasmodic effects of ethyl acetate and aqueous residual fractions of *Diospyros mespiliformis* Hochst. ex A. DC (Ebenaceae) leaves on isolated duodenum of rat. *Journal of Drug Delivery and Therapeutics*, *13*(12), 148-154.
43. Nunes, C. D. R., Barreto Arantes, M., Menezes de Faria Pereira, S., Leandro da Cruz, L., de Souza Passos, M., Pereira de Moraes, L., ... & Barros de Oliveira, D. (2020). Plants as sources of anti-inflammatory agents. *Molecules*, *25*(16), 3726.
44. Oguntibeju, O. O. (2018). Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. *Journal of inflammation research*, 307-317.
45. Okoli, R. I., Turay, A. A., Mensah, J. K., & Aigbe, A. O. (2009). Phytochemical and antimicrobial properties of four herbs from Edo State, Nigeria. *Report and opinion*, *1*(5), 67-73.
46. Okwuosa, C. N., E.N. Shu, N.C. Azubike, D.C. Nwachukwu, A.C. Onuba, I.N. Nubila, F.C. Otuu and I.J. Chukwu. 2016. Anti-diarrhoeal properties of the leaf extracts of *Combretum racemosum*. Beauv in rodents. *Der. Pharma. Lett*., 8 (18): 207-213.
47. Olalekan, M. A. (2023). Comparative Phytochemical and Antioxidant Analysis of the Leaf Extracts of Two Nigerian Medicinal Plants. *Journal of Science and Mathematics Letters*, *11*(1), 30-38
48. Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and chemical toxicology*, *46*(7), 2326-2331.
49. Ouahhoud, S., Marghich, M., Makrane, H., Karim, A., Khoulati, A., Mamri, S., ... & Saalaoui, E. (2023). In vitro assessment of myorelaxant and antispasmodic effects of stigmas, tepals, and leaves hydroethanolic extracts of Crocus sativus. *Journal of Food Biochemistry*, *2023*.
50. Packirisamy, S., Gunam, V., Mahendra, J., Rajendran, D., & Rajagopal, P. (2023). Preliminary Phytochemical screening and Antioxidant properties of Methanolic root extract of *Picrorhiza kurroa*. *Research Journal of Pharmacy and Technology*, *16*(9), 4266-4270.
51. Pearson, D. 1976. Chemical analysis of food.Churchill livington, Edinburgh, UK.103110 pp.
52. Pereira, R. B., Rahali, F. Z., Nehme, R., Falleh, H., Jemaa, M. B., Sellami, I. H., ... & Pereira, D. M. (2023). Anti-inflammatory activity of essential oils from Tunisian aromatic and medicinal plants and their major constituents in THP-1 macrophages. *Food Research International*, *167*, 112678.
53. Peri, C and C. Pompei. 1971. Estimation of different phenolic groups in vegetable extracts. *Phytochem*., 10(9): 2187-2189.
54. Radovanović, K., Gavarić, N., & Aćimović, M. (2023). Anti-Inflammatory Properties of Plants from Serbian Traditional Medicine. *Life*, *13*(4), 874.

55. Rauf, A., Jehan, N., Ahmad, Z., & Mubarak, M. S. (2017). Analgesic potential of extracts and derived natural products from medicinal plants. *Pain Reli-From Analg to Altern Ther Rijeka: InTech*, 339-51.
56. Revathi, M., Manonmani, R., & Sowmiya, V. (2024). PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF NELUBO NUCIFERA GAERTN. DOI: 10.20959/wjpr20247-31881
57. Sadiq, A., Zeb, A., Ullah, F., Ahmad, S., Ayaz, M., Rashid, U., & Muhammad, N. (2018). Chemical characterization, analgesic, antioxidant, and anticholinesterase potentials of essential oils from *Isodon rugosus* Wall. ex. Benth. *Frontiers in pharmacology*, *9*, 623. DOI: Org/10.3389/fphar.2018.00623
58. Savithramma, N., Rao, M. L., & Suhrulatha, D. (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research*, *8*(3), 579-584
59. Senguttuvan, J., Paulsamy, S., & Karthika, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. *Asian Pacific journal of tropical biomedicine*, *4*, S359-S367.
60. Sharifullah, S. M., Khan, I., Ali, S., Ali, K., & Kumar, T. (2016). Study of important medicinal plants of district Dir Upper. *J. Weed Sci. Res*, *22*(4), 595-606.
61. Shoaib, G., Shah, G. M., Hussain, M., Muhammad, S., Rehman, I. U., Khan, A., & Shah, M. (2017). Ethnomedicinal plants and traditional knowledge of some Phenorogams of lower Kaghan valley, district Mansehra, Pakistan. *J. Appl. Environ. Biol. Sci*, *7*(5), 21-28.
62. Shoaib, G., Shah, G. M., Shad, N., Dogan, Y., Siddique, Z., Shah, A. H., & Nedelcheva, A. (2021). Traditional practices of the ethnoveterinary plants in the Kaghan Valley, Western Himalayas-Pakistan. *Revista de Biología Tropical*, *69*(1), 1-11.
63. Sun, Y.-X., and J. -C. Liu (2011). Chemical constituents and biological activities of *Euphorbia fischeriana* STEUD. Chemistry and Iodiversity 8 (7): 1205–1214.
64. Sunil, M., Vedavijaya, T., Sayana, S. B., & Podila, K. S. (2023). Phytochemical Analysis and Antioxidant Evaluation of the Ethanolic Extract of the Leaves of *Abutilon indicum*. *Cureus*, *15*(10).
65. Tadesse, E., E. Engidawork, T. Nedi and G. Mengistu. 2017. Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. *BMC compl. Alter. Med.*, 17(1): 190.
66. Ullah, N., Haq, I. U., Safdar, N., & Mirza, B. (2015). Physiological and biochemical mechanisms of allelopathy mediated by the allelochemical extracts of *Phytolacca latbenia* (Moq.) H. Walter. *Toxicology and industrial health*, *31*(10), 931-937.
67. Winter, C.A and C.C. Poster. 1957. Effect of alteration in side chain up on anti-inflammatory and liver glycogen activities in hydrocortisone ester. *J. American Pharmacol. Soc*., 46:515-519.
68. Yasmeen, M., Prabhu, B., & Agashikar, N. (2010). Evaluation of the antidiarrhoeal activity of the leaves of *Ixora coccinea* Linn. in rats. *Journal of clinical and diagnostic research*, *4*(5), 3298-3303.
69. Ybañez-Julca, R. O., Pino-Ríos, R., Quispe-Díaz, I. M., Asunción-Alvarez, D., Acuña-Tarrillo, E. E., Mantilla-Rodríguez, E., ... & Benites, J. (2023). Antispasmodic Effect of *Valeriana pilosa* Root Essential Oil and Potential Mechanisms of Action: Ex Vivo and In Silico Studies. *Pharmaceutics*, *15*(8), 2072.
70. Zare, A., Khaksar, Z., Sobhani, Z., & Amini, M. (2018). Analgesic effect of Valerian root and turnip extracts. *World Journal of Plastic Surgery*, *7*(3), 345. Doi: 10.29252/wjps.7.3.345