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Botanical Identity, Bioactive Principle, Antipyretic, Analgesic and Anti inflammatory Activities of *Rungia repens* (L) Nees

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ABSTRACT

In this study we aimed to fix *Rungia repens* (L) Nees botanical identity, macroscopical, microscopical diagnostic characters and to isolate the bio-active principle responsible for biological actions shown by ethyl acetate and methanol extracts. Bioactive compounds were isolated by running column chromatography, characterized and percentage of activity of bioactive principle was calculated. Microscopical study reveals the characteristic nature of stomata, trichome, leaf mid rib and stem. The extracts were found to be non toxic upto the dose of 3000 mg/kg of *Rungia repens* ethyl acetate extract (RREAE) and methanolic extract (RRME) respectively. RREAE and RRME produced a significant and dose dependent inhibition of analgesic, anti inflammatory and antipyretic activity. Bioactive principle isolated was a flavonoid luteolin shows maximum activity equal to that of standard drug. We believe that the antipyretic, analgesic, and anti-inflammatory activity of the RREAE and RRME were due to its bioactive principle flavonoid luteolin present in the extract.

Keywords: *Rungia repens*, quantitative microscopy, anti pyretic, analgesic, anti inflammatory and luteoline

1. INTRODUCTION

Pyrexia may be a result of secondary impact of infection or one of the consequence of tissue damage, inflammation, graft rejection and malignancy or other diseased status. Pyrexia was enhanced by formation of cytokines (IL 1β, IL-6, Interferon α, β and TNFα). This pro-inflammatory mediator increases the synthesis of PGE_2 in many tissues near to the pre-optic hypothalamic area via increase in cyclic AMP and triggers the hypothalamus to elevate the normal body temperature by promoting increase in heat generation and decrease in heat loss.[1-3] Inflammation is a homeostatic phenomenon.[4] Inflammation is usually associated with pain as a secondary process resulting from the release of analgesic mediators.[5,6] When inflammation is left to itself, it displays a short course of reaction (acute inflammation), but under certain conditions it becomes a more sustained event (chronic inflammation) such as that prevailing in human rheumatoid arthritis. A vast array of substances, the so-called mediators of inflammation, are formed or released either concurrently or successively at the site of injury from various plasma or cell sources in response to an etiological factor.[7]

During the course of reviewing the traditional medicinal plants in various states of India, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs.[8] It was seen that many plants have remarkable effects as on pyrexia, pain, infections, and liver ailments.

When used as an aqueous extract, churna, or arista. During a appraisal of medicinal plants, it was found parpata or parpatha or parpadagam was mentioned in different formulations mainly for its antipyretic effect.

While trying to fix its botanical source it was observed that parpata (or) parpatha (or) parpadagam has been given different botanical sources by different authors.[9-11] From the review, it was very clear the plant name parpata, parpatha, and parpadagam indicate a remedy used for pyrexia, pain and infections. The term parpatha in Sanskrit and kodagasalai in Tamil was considered as a potential plant for its antipyretic effect. Hence, *Rungia repens* (L) Nees was taken up to study its botanical identity to establish standards in agreement with the WHO for the vegetable drugs, isolation of bioactive principle having an effect on pyrexia, pain and bacterial infections based on the pharmacological effect shown by alcohol extract.

Rungia repens (L) Nees (Acanthaceae) is found growing as a shade loving weed in moist places, sides of water channels, bunds of paddy fields, and also under the shadow area of coconut trees. It is an annual herb; stem is decumbent often rooting near the base, attaining a height of about 25-45cm. The flowering and fruiting time of *Rungia repens* (L) Nees is generally during winter season from July to December with flowering reach a peak during November. The plant is known in Hindi as Kharmor, Sanskrit as parpatha and in Tamil as Kodagasalai. The aerial parts of the plant have been dried and pulverized for use in the treatment of cough and fever; it also credited with vermifugal and diuretic properties.[9] Fresh bruised leaves are mixed with castor oil and applied on scalp to cure tinea capities, a scaly fungoid infection usually occurring amongst children.[12] Investigation on the flavonoid pigments in ivory-white and pale yellow flowers showed the presence of luteolin and chrysoeriol (3'-*O*-methyl luteolin) and their glucosides.[13,14] Since no detailed previous work relating to the pharmacognostical studies of the drug has been brought on record. It was decided to make a thorough and detailed study to identify the drug. A detailed quantitative microscopy and linear measurements were also carried out. To confirm its pharmacological activity, compounds were isolated and characterized systematically.

2. MATERIALS AND METHODS

2.1 Plant collection, Drying, pulverizing and preparation of extract

The *Rungia repens* (L) Nees herb was collected from the moist places near Madurai, identified and authenticated by Mr.V.Chelladurai Research Officer of Botany, Central Council for Ayurveda and Siddha, Government of India.

The voucher specimen was preserved in our laboratory for future reference. After collection of the plant, the root was removed; the aerial part was washed thoroughly in tap water and dried in shade for about 10 days under controlled temperature (25± 2 ºC), powdered and passed through a 40 mesh sieve and stored in a well closed container for further use. Coarsely powdered, dried aerial plant (1.2 kg) was successively soxhlated using petroleum ether, chloroform, ethyl acetate and methanol for 72 h at room temperature respectively. The extracts were filtered and the solvents evaporated to dryness under reduced pressure in an Eyela rotary evaporator at 40 to 45 ºC. The percentage yield was noted as 13 for petroleum ether, 3.1 for chloroform, 5.4 for ethyl acetate and 7.9 for methanol. Preliminary phytochemical screening were performed.[15]

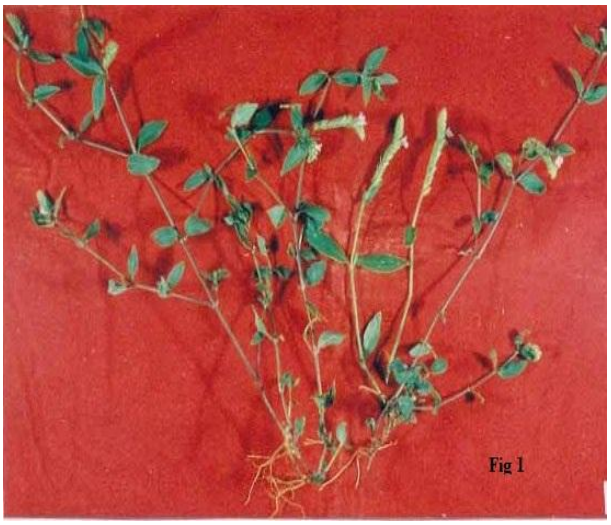

Fig 1: picture depecits the whole plant of *Rungia repens* (L) Nees

2.2 Chemicals and Instruments

Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. TLC was carried out using Merck aluminium sheet coated with silica gel GF_{254} (0.2 mm). The Melting points were recorded in a Technico melting point apparatus. The UV Spectrum was measured with a Shimatzu UV-1700 double beam Spectrophotometer. The IR spectra of isolated compounds were recorded on a Jasco FTIR-4100 in potassium bromide discs. ^1H NMR spectra were recorded on a Bruker 300 FT NMR Spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Mass studies were done by JEOL SX-102 instrument.

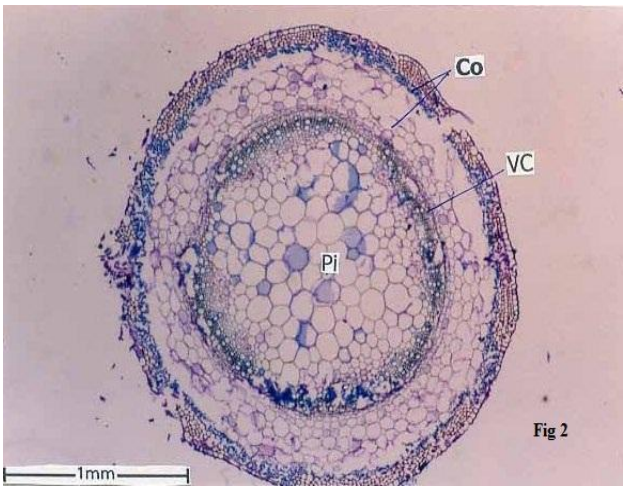

Fig 2: TS of stem (Co- Cortex and Pi-Pith)

2.3 Morphology and anatomical study of the plants

Morphology of the entire plant was studied.[16,17] Leaf, stem and root of *Rungia repens* were studied as per the described method.[18-22]

2.3.1 Preparation of specimens

Leaf, stem and root parts of *Rungia repen* were fixed using fixing agent FAA (Formalin (5 ml) + Acetic acid (5 ml) + 70% ethyl alcohol (90 ml) for 24 h. Then, the specimens were dehydrated with graded series of *tert*-butyl alcohol (TBA).[19] Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 ^{o}C) until TBA solution attained super saturation. The specimens of leaf, stem, and root were casted into paraffin blocks.

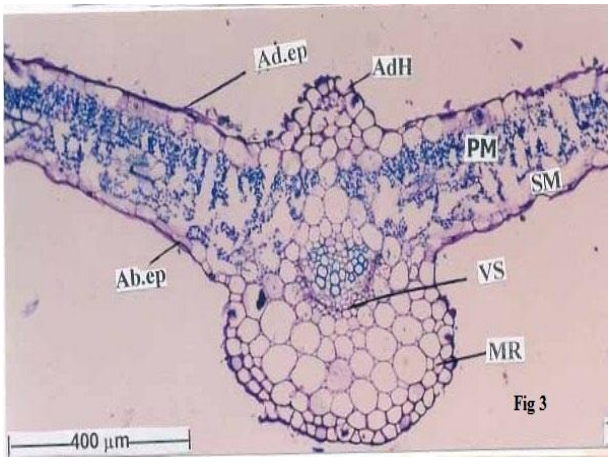

Fig 3: TS of mid rib and lamina of leaf (ABE- Abaxial epidermis; Ade-Adaxial epidermis; Ads-Adaxial side; La-Lamina; MR-Midrib; Ph-Phloem; PM-Palisade mesophyll; SM-Spongy mesophyll; VS-Vascular strand; X-Xylem)

2.3.1.1 Microscopical Sectioning

The paraffin embedded specimens of leaf; stem and roots were sectioned with the help of rotary microtome to a thickness of 10-12 µm. The leaf, stem and root embedded in the wax was cut and removed along with wax. The material in the wax cube was mounted on a wooden piece and then clamped into the microtome.[20]

2.3.1.2 Staining

The sections were first dewaxed by customary procedure.[20] The sections were stained with toludine blue (0.25% having a pH 4.7) a polychromatic stain to confirm the nature and presence of cellulose walls, lignified cells, suberin, mucilage and proteins where the dye will render pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies. Sections were also stained with safranin and fast-green and iodine in potassium iodide to confirm the presence of starch. For studying the stomata morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) was treated with 5% sodium hydroxide.[19]

2.3.1.3 Photomicrographs

In order to supplement the descriptive part, the photomicrographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the purpose of studying calcium oxalate crystals, starch grains and lignified walls photograph was taken under polarized light microscope. Since these structures have shinning property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.[21,22]

2.3.1.4 Powder microscopy

The aerial part of dried plant materials of *Rungia repens* was powdered and immersed in the Jeffrey's maceration fluid.[20] The fluid consists of equal volumes of 5% chromic acid and 5% nitric acid. The fluid with the materials was kept at 55ºC for 5 h. Then the material is washed thoroughly with water and placed on a glass slide in a drop of safranin (0.5%) for 20 min. The strain was drained carefully and mounted with a drop of dilute glycerin. The cells are spread well with a needle and the material is covered with cover slip. The slide thus prepared was examined under the microscope to study different components of the macerate.

2.4 Physicochemical parameters

Loss on drying, ash value, sulphated ash, quantitative microscopy and extractive values were determined as per the standard procedures

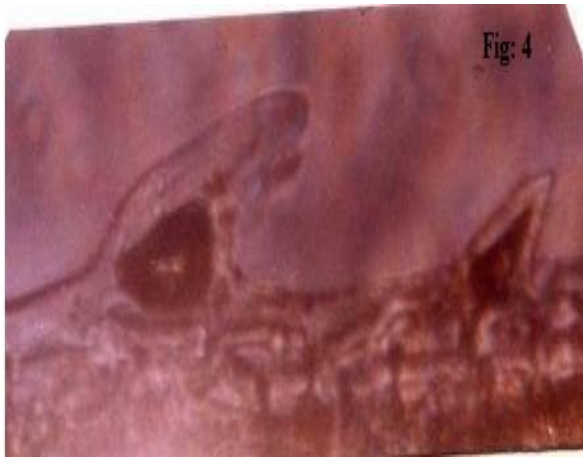

Fig 4: Conical trichome of leaf epidermis

2.5 Pharmacological screening

2.5.1 Experimental animals

Wistar albino rats and Swiss albino mice were used for pharmacological evaluations. The animals were maintained in colony cages at 25±2 °C, relative humidity of 45-55%, under a 12 h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use. The Institutional Animal Ethics committee approved the protocol adopted for the experimentation of animals.

2.5.2 Acute toxicological studies

Acute oral toxicity was performed as per the OECD 423 guidelines.[23] Three female Albino mice weighing 22–25 g were used in the study. The animals were fasted over night before the treatment. Each animal was administered orally a dose level of 5 mg/kg body weight by gastric intubation. The administered dose was assigned as toxic if mortality was observed in three animals. The same dose was repeated again if mortality was observed in one animal to confirm the toxic dose. If no mortality was observed, the procedure was repeated with further higher doses such as 50, 300, 1000, 2000 and 3000 mg/kg body weight.

2.5.3 Antipyretic activity

Antipyretic activity was determined on male Wistar albino rats, weighing about 200-250 g. The rats showing 37.5 ± 0.5 ºC were selected and then they were fasted for 24 hours before inducing pyrexia. Their normal body temperature was recorded. The normal body temperature of each rat was measured rectally at predetermined intervals. Fever was induced by injecting subcutaneously 15% w/v 10 ml/kg yeast suspended in 0.5% w/v sodium carboxy methyl cellulose (CMC) solution into the animal's dorsum region and injected site is massaged in order to spread and they were allowed to food.[24] After advocating this presumed dose uniformly, the rats were returned to their housing cages. After 19 h of yeast injection, the rectal temperature of each rat was measured again using a digital thermometer. Only rats that showed a net increase in temperature of at least 0.7 ºC were used for the antipyretic study. The animals were divided into 7 groups of 6 each and numbered. The control, standard and test substances were given to the animals by gastric tube. After the drug was administered, the temperature of all the rats in each group was recorded at an interval of 1 h, 2 h, 3 h and 4 h. The mean temperature was found out for each group and compared with the standard drug values. The animals marked as group -I received orally 5 ml/kg of body weight of 0.5% CMC. The animals marked group-II, group III, group, IV group V, group VI and group VII received orally 150 mg/kg of paracetamol in 0.5% CMC, 300 mg/kg, 600 mg/kg of RREAE, 300 mg/kg, 600 mg/kg of RRME, and isolated compound 10 mg/kg respectively.

2.5.4 Analgesic activity

The test extracts and the standard drugs were administered in the form of a suspension (0.5% CMC) by oral route. Test for analgesic activity was performed by tail-flick technique on Swiss albino mice (25-35 g) of either sex selected by random sampling technique.

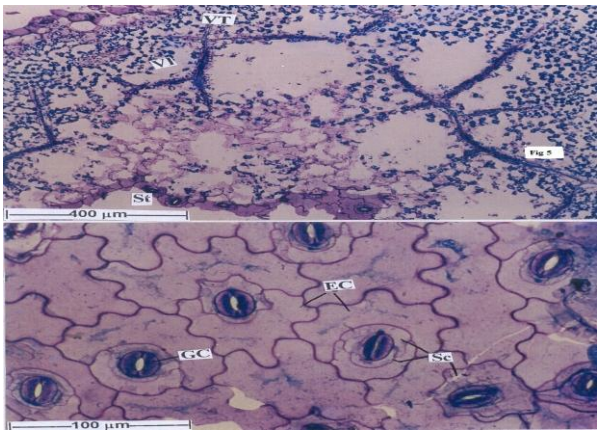

Fig 5: Surface view of the epidermis with diacytic stomata (GC-Guard cells; Sc-Subsidiary cells; St-Stomata; VI-Vein islet;VT-vein termination)

Table 1: Antipyretic activity of the extracts of *Rungia repens*

Groups	-18 h	0 h	30 min	1 h	2 h	3h
Group I-Control	35.00± 0.44[a]	37.00± 0.36[a]	37.33± 0.33[a]	38.00± 0.25[a]	38.50± 0.34[a]	39.58± 0.20[a]
Group II: Paracetamol(150mg/kg)	33.83± 0.54[a]	38.50± 0.25[a]	37.50± 0.40[a]	36.75± 0.35[a]	36.33± 0.65[a]	35.67± 0.44[a]
Group III: RREAE 300 mg/kg	35.33± 0.42[a]	38.00± 0.44[a]	37.75± 0.44[a]	37.00± 0.36[a]	36.83± 0.40a	36.92± 0.37[a]
Group IV: RREAE 600 mg/kg	34.00± 0.36[a]	38.33± 0.33[a]	37.83± 0.40[a]	37.00± 0.25[a]	36.33± 0.21[a]	36.17± 0.16[a]
Group V: RRME 300 mg/kg	35.33± 0.33[a]	37.83± 0.27[a]	37.17± 0.30[a]	36.67± 0.21[a]	36.33± 0.21[a]	36.17± 0.16[a]
Group VI: RRME 600 mg/kg	34.33± 0.33[a]	38.17± 0.30[a]	37.17± 0.30 [a]	36.50± 0.22 [a]	36.00± 0.25[a]	35.67± 0.21[a]
GroupVII: Luteolin 10 mg/kg	34.50± 0.34[a]	38.50± 0.22[a]	37.33± 0.33 [a]	36.33± 0.21 [a]	35.83± 0.30 [a]	35.17± 0.30 [a]

Data expressed as mean ± SEM from six different experiments. Significance levels [a]p<0.001 as compared with the respective control

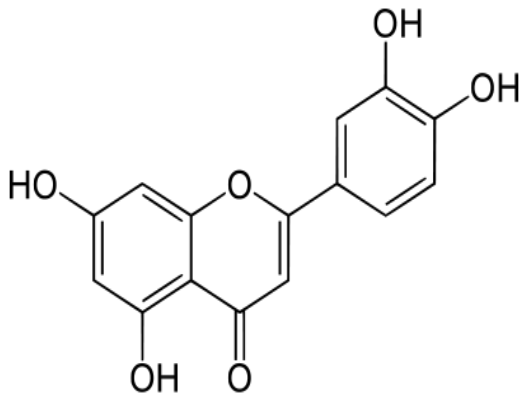

Fig 6: Isolated compound-Luteolin [2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone]

Each group consisted of six animals.[25] Diclofenac sodium at a dose level of 5 mg/kg and 10 mg/kg was administered orally as reference drug for comparison. The test extracts at two dose levels (300, 600 mg/kg) were administered orally. The reaction time was recorded at 30 min, 1, 2 and 3h after the treatment, and cut-off time was 10 sec. The percent analgesic activity (PAA) was calculated by the following formula; PAA = $(T_2 - T_1) / (10 - T_1)$ X 100; Where T_1 is the reaction time (s) before treatment and T_2 is the reaction time (s) after treatment.

2.5.5 Anti-inflammatory activity

The inflammatory reaction is readily produced in rats in the form of paw edema using carrageenan. The rats were divided into seven groups, each consisting of six animals. RREAE and RRME of (300 and 600 mg/kg in 0.5 % w/v CMC was administered orally. The first and second groups received 5 ml/kg of 0.5 % w/v CMC as vehicle control and 10 mg/kg (p.o) of diclofenac sodium as standard respectively, for comparative pharmacological assessment. Group-III, IV, V, VI and VII treated with 300 and 600 mg/kg of RREAE and RRME and isolated compound respectively. After 30 min of extracts administration, 0.1 ml of 1% w/v carrageenan was injected into the right hind paw sub-plantar region of each rat. The left paw served as reference (non-inflammatory paw) for comparison. The paw volumes of both paws of control and extract treated rats were measured at 1 h, 2 h, 3 h, 4 h and 5 h after carrageenan administration (Winter et al., 1962). The percentage inhibition for each rat and each group was obtained by using the formula C−T/C×100 where C is the edema rate of control group and T for treated group.

3. RESULTS AND DISCUSSION

3.1 Morphological study of the plants

The stem was slender, cylindrical, and decumbent and erect (Fig 1). The leaves are shortly petiolate. Petiole-0.1 to 0.3 cm, leaves are having the length-0.7 - 4.5cm and width 0.3-1.3cm, Shape- lanceolate, Apex-acute. Inflorescence: Spikes- terminal 3-7 cm.

3.2 Microscopy of stem

Epidermis consists of single layer of tangentially elongated cells and cortex is made up of 3 to 4 layers of collenchymas followed by parenchyma (Fig 2). Endodermis with a thick radial wall is followed by phloem. Pericycle is absent. Phloem is followed by Xylem. Xylem tissue is made up of xylem parenchyma, vessels and meduallary ray cells, which is made up of lignified parenchyma. In the pith, there are certain closely arranged polygonal cells followed by large parenchymatous tissue with inter cellular spaces. In a longitudinal sectional view, an epidermis made up of single layer of compactly arranged rectangular cells with a smooth cuticle were seen. Uniseriate multicellular trichomes are seen. This is followed by zone of cortex consisting of 2-4 layers of elongated collenchyma followed by two layers of elongated

chlorenchyma, and 2-3 layers of parenchyma. Phloem is made up of compactly arranged elongated parenchymatous cells and sieve tissue. Xylem vessels are lignified and are pitted, reticulated, spiral and annular. Pith is composed of elongated large parenchymatous tissue with intercellular spaces.

3.3 Microscopy of leaf

The midrib is having a slight projection on the upper side and the lower side was wider with centrally placed vascular bundle with radiating rows of xylem and narrow phloem (Fig 3). Below, the upper epidermis in the projection area 2-3 layers of collenchymas tissue, the palisade cells are discontinuous in the middle. In the lower side, inner to the lower epidermis 1-2 layers of collenchyma tissue. Above the collenchymas, loosely arranged large parenchyma cells are surrounding the vascular bundle. In the lamina part, a single layer of palisade cells followed by spongy parenchyma. Conical warty trichomes (Fig 4) in the surface view of the epidermis with diacytic stomata were seen (Fig 5).

3.4 Powder microscopy

The powdered aerial parts showed the unicellular conical trichomes, collenchymatous cells, palisade cells, uniseriate multicellular trichomes, unicellular conical warty trichomes and wavy anticlinal walls with diacytic stoma. Fragments of lignified spiral, reticulate and pitted vessel elements, a gland tipped trichomes were also seen.

3.5 Quantitative Microscopy and linear Measurements

In the present work, parameters of analytical microscopy including stomatal number, stomatal index, vein islet and vein termination number of *Rungia repens* (L) Nees had been investigated. Customary methods of analytical microscopy as described by Wallis (1967) were followed and the numerical values were recorded. Stomatal number UE 3 - 3.8 -5μm; for LE 5- 8.3 - 11μm(Fig 4); Stomatal Index UE 12.00 - 17.3 - 22.72μm; for LE 17.85 - 25.82 - 32.14μm, Vein-islet number 4- 5.5 -8μ; Vein termination number 5- 9.2 -12μ. The Length and width of the trichome (Fig 5) in the leaf was measured by using the same methods as described elsewhere and recorded. Unicellular conical warty trichomes length 79.8- 119.7 - 159.6μ; Width 13.3 - 19.9 - 26.6μ; Two cell convering trichomes length 39.9 - 93.1 -146.3μ ; width 13.3 - 13.3 - 13.3μ; Unicellular slightly bent conical trichomes length 26.6 - 59.85 - 93.1μ ; Width 13.3 - 19.5 - 26.6μ ; Elongated sharp Trichomes length 93.1 - 126.3 -159.6μ ; width 13.3 -13.3 -13.3μ ; Multicellular uniseriate trichomes length 159.6 - 239.4 -319.2μ ; Width 13.3-19.95-26.6μ .

3.6 Physiochemical parameters

Physical studies of *Rungia repens* powder showed the values of percentage yield in Loss on drying (5.30), Total Ash (13.98), Acid Insoluble ash (1.80), Water soluble ash (8.83) and Sulphated ash (10.38). The extractive values in percent yield were found to be 1.3 for petroleum ether extract, 3.1 for chloroform extract, 5.4 for ethyl acetate extract and 7.9 for ethanol extract.

3.7 Pharmacological Activity

3.7.1 Acute toxicity

The animals were observed for toxic symptoms of behavioral changes, locomotion, convulsions and mortality. The extracts found to nontoxic upto the dose of 3000 mg/kg**.**

3.7.2 Anti pyretic activity

Ethyl acetate and methanol extracts of the *Rungia repens (L) Nees* were screened for antipyretic activity (Table 2). Methanol extract at the dose of 600 mg/kg body weight showed maximum antipyretic activity response (P<0.001) equal to that of paracetamol as standard. The results indicated that the major component responsible for antipyretic activity may be present in the methanol extract.

3.7.3 Analgesic activity

The results of analgesic testing indicate that the test extracts exhibited moderate analgesic activity at 30 min of reaction time and an increase in activity at 1 h which reached a peak level at 2 hr. Decline in activity was observed at 3 h Table 2. Among the extracts, methanol extract shown the most potent activity which is equipotent to that of reference diclofenac sodium. In the tail flick method, the increase in latency period at different time points significantly differed (P<0.01) compared to baseline values within the same drug treated groups. The extract and Diclofenac sodium caused significant increase (P<0.001) in the percentage reaction time whilst the control and lower dose of extract (300 mg/kg) caused no change. The percentage increase in reaction time was dose dependent. At all the specified time intervals, the percentage of tail flick elongation time differed significantly (P<0.001) between the extract and Diclofenac sodium at both the doses of plant extract, being greater for Diclofenac sodium. At the peak of activity, 300mg/kg and 600mg/kg extract showed 43.66% (P<0.001) and 46.76% (P<0.001) percentages of tail flick elongation time respectively, whilst Diclofenac sodium gave 80.08% (P< 0.001) elongation of tail flicking time Table 2. Time to reach peak activity was same (+30 min) for the extract and Diclofenac sodium.

3.7.4 Anti inflammatory activity

The results for the anti-inflammatory activity of RREAE tested by carrageenan induced rat paw edema were recorded in Table 2. RREAE and RRME at a dose of 300 mg/kg showed 22.88% and 24.78% protective effect respectively. RREAE and RRME at a dose of 600 mg /kg showed a significant protective effect (23.61% and 25.96%) of paw edema. The standard drug diclofenac sodium (10 mg/kg) produced maximum protective effect of 33.04%. Thus RREAE and RRME exhibited a dose dependent inhibition on carrageenan-induced rat paw oedema Table 2.

3.8 Confirmation of bioactive isolated active principle

Fractionation in column chromatography sorted out the bioactive molecule known to be leutolin (Fig: 6), which possess the best pharmacological activity. The H^1-NMR investigation has given the following chemical shifts (δ in ppm). 5.12 (s, 1H, OH), 5.45 (s, 1H, OH), 5.70 (s, 1H, OH), 5.99 (s, 1H, OH), 6.31-7.59 (m, 6H, Ar-H). The M^+ peak appeared at 286 confrimed as leutolin by the molecular mass of the compound in mass spectrum and IR (KBr) cm-1: 3513, (OH), 3096 (Ar-CH), 1734 (C=O), 1032 (C-O-C). The molecular formula was found to be $C_{15}H_{10}O_6$.

4. CONCLUSION

Pyrexia may be due to the infection or one of the sequence of the tissue Damage, inflammation, graft rejection or other diseased states.[26] The enhanced formation of pro inflammatory mediators like cytokines, interleukin 1β, α, and TNF – α) increases the synthesis of prostaglandin E_2 (PGE_2) near the pre-optic hypothalamus area, and leads to triggering the hypothalamus to elevate the body temperature due to the infected or damaged tissue.[27] Paracetamol a non-steroidal anti-inflammatory drug (NSAID), is commonly prescribed to treat fever inhibits cyclo-oxygenase -2 (COX-2) to decrease the body temperature by inhibiting PGE_2 biosynthesis. It causes toxic effects to the liver cells, glomeruli, cortex of brain and heart muscles due to inhibition of COX-2, but natural COX-2 inhibitors have lower selectivity with fewer side effects.[28] The antipyretic activity demonstrated that RREAE and RRME possess a significant effect in maintaining normal body temperature and reducing Brewer's yeast induced pyrexia in rats, and their effects, are comparable with that of standard antipyretic drug paracetamol. Such reduction in rectal temperature of tested animals by both the extracts at 300 mg/kg and 600 mg/kg may be due to the presence of flavonoids. The presence of flavonoids in RREAE, RRME and the presence of isolated luteolin, may be responsible for antipyretic activity. The antipyretic activity of luteolin isolated from other medicinal plants was already established and the activity was attributed due to the inhibition of synthesis of TNF-α and anti-oxidant activity associated with amelioration of inflammatory actions of cytokines.[29] It was also evident from the study that the antipyretic activity of RREAE at 600 mg/kg is almost similar to that of paracetamol. Thus the antipyretic activity supported the claims of traditional practitioners of *Rungia repens* as an antipyretic remedy.

The present study indicates that the methanol extract has shown significant analgesic action in mice, by increasing the latency period in the tail flick test. Preliminary phytochemical screening revealed the presence of flavonoids compound in *Rungia repens* and they are recognized as powerful antioxidants. In the analgesic activity, flavonoids primarily target prostaglandins which are involved in controlling pain perceptions.[30] Therefore, it can be assumed that *Rungia repens* extracts might suppress the formation of prostaglandins by inhibiting or antagonizing the enzyme cyclooxygenase and also makes the nociceptors more sensitive to pain producing agents such as bradykinin and ultimately relieve the sensation of pain.[31] Carrageenan induced paw oedema method is the most commonly used method for screening and evaluation of anti-inflammatory activity of natural and synthetic compounds. The initial phase of study observed at 1 h may be attributed due to the release of histamine and serotonin.[32] The oedema maintained during the plateau phase is presumed to be due to the release of kinin like substances.[33,34] The second accelerating phase of swelling may be due to the release of prostaglandins i.e., PGE_2 and nitric oxide.[35] During the inflammatory phase, from the macrophage, the reactive free radical nitric oxide (NO) is synthesized by inducible NO synthase (NOS), where the excessive production of NO plays a pathogenic role both in acute and chronic inflammations.[36] NO is reported for vasodilatations, increase in vascular permeability, oedema formation and inducing synthesis of prostaglandins at the site of inflammation.[37,38]

RREAE and RRME showed significant anti inflammatory activity at 3 h against carrageenan injection, suggesting that it predominantly inhibited the release of inflammatory mediators from phlogistic stimuli. The anti-inflammatory activity may be due to presence of flavonoids in RREAE and RRME, confirmed by phytochemical investigations and the compound luteoline isolated from the column fractions. In the present study, flavonoids of *Rungia repens* was found to suppress carrageenan induced oedema significantly ($P<0.01$) but its anti-inflammatory action was less effective than diclofenac sodium. 5-Hydroxytryptamine (5-HT), bradykinin and prostaglandins have been identified as mediators for carrageenan hind paw edema. Earlier study of flavonoids from Acanthaceae family reduced significantly 5-HT and bradykinin induced hind paw oedema. Hence, flavonoids can be safely be interpreted that the extract of *Rungia repens* was antagonistic to 5-HT or bradykinin or both.

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We believe that the antipyretic, analgesic and anti-inflammatory activity of the RREAE and RRME were due to its bioactive principle flavonoid luteolin present in the extract. Our results contribute towards validation of traditional plant with sanskrit name parpatha and botanical name mentioned by many authors as *Rungia repens* (L) Nees in the treatment of pain, fever and inflammation.

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Table 2: Anti-inflammatory and Analgesic activity of the extracts of *Rungia repens*

Group/Treatment	Percent Anti-inflammatory activity				Percent Analgesic activity			
	30 min	1 h	2 h	3h	30 min	1 h	2 h	3h
Group I-Control	---	----	---		1.85 ± 1.85 ^{Ns}	5.78 ± 2.59 ^{Ns}	7.87 ± 2.50 ^{Ns}	3.93 ± 2.49 ^{Ns}
Group II: Diclofenac sodium (10mg/kg)	32.45 ± 9.83 ^{b}	36.50± 4.33 ^{c}	43.78 ± 3.69 ^{c}	33.04 ± 2.97 ^{b}	35.18 ± 3.89 ^{c}	42.82 ± 5.20 ^{c}	48.61 ± 5.83 ^{c}	29.17 ± 3.42 ^{c}
Group III: RREAE 300 mg/kg	22.80±5.87 ^{a}	32.93±5.58 ^{c}	38.95±2.35 ^{c}	22.88±2.98 ^{a}	25.49 ± 2.48 ^{c}	29.41 ±4.29 ^{c}	33.33 ±3.92 ^{c}	17.64 ± 3.03 ^{b}
Group IV: RREAE 600 mg/kg	25.43±6.57 ^{a}	37.30±5.31 ^{c}	43.38±5.04 ^{c}	23.61±2.58 ^{a}	35.30±2.63 ^{c}	37.26±2.48 ^{c}	41.18±3.03 ^{c}	20.46±4.19 ^{c}
Group V: RRME 300 mg/kg	27.19±4.78 ^{c}	36.50±5.45 ^{c}	41.18±2.01 ^{c}	24.78±2.79 ^{c}	29.41±3.03 ^{c}	35.30±2.63 ^{c}	39.22±3.61 ^{c}	21.57±3.92 ^{c}
Group VI: RRME 600 mg/kg	30.70±6.28 ^{c}	42.46±4.81 ^{c}	49.60±2.12 ^{c}	25.96±2.64 ^{c}	43.14±3.61 ^{c}	49.02±2.47 ^{c}	52.94±3.03 ^{c}	33.33±2.48 ^{c}
Group VII: Luteolin 10 mg/kg	35.08±4.43 ^{c}	46.82±5.45 ^{c}	53.82±5.48 ^{c}	27.42±2.37 ^{c}	45.10±3.92 ^{c}	52.94±4.29 ^{c}	54.90±3.61 ^{c}	35.30±2.63 ^{c}

Data expressed as mean ± SEM from six different experiments. Significance levels $^{a}p<0.05$, $^{b}p<0.01$and $^{c}p<0.001$ as compared with the respective control

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