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Analysis of anti-oxidant and anti-inflammatory potential of *Baccaurea courtallensis* (Wight) Mull. Arg

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Abstract
The ethanolic leaf extracts of *Baccuarea courtallensis* (Wight) Mull.Arg. a member of the family Euphorbiaceae were assessed for its phytochemical, antioxidant and anti-inflammatory potential. The preliminary screening showed the presence of different phytochemicals among them phenol and flavonoid were quantified. The extract showed a significant inhibition of carrageenan-induced rat paw edema in Wistar rat compared to the standard anti-inflammatory drug indomethacin. Antioxidant activity was evaluated through (*in vitro*) assays such as DPPH radical scavenging assay and total antioxidant capacity by phosphomolybdenum method.

Keywords: DPPH, carrageenan, indomethacin, phosphomolybdnum, *Baccaurea courtallensis*

1. Introduction
Health is a major asset which is as important as food and shelter. This is mainly ruined by diseases. Diseases are biological conditions which are characterized as abnormalities in the function or the structure of certain organs or entire organ systems. To maintain the health, man invented the remedy medicine for disease from the early days itself. The major source for medicines are the "Plants" which are used in the early days in the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations. The understanding of the use of medicinal plants for remedies is accumulated in to a traditional knowledge of health care. Traditional knowledge was not scientifically proven in early days but these were later used for the development of herbal medicines. Nowadays, herbal medicines are the one that are used widely for primary health care. Greater acceptability and response to human body make these drugs more valuable. Several herbal drugs were marketed without scientific validation.
Baccaurea courtallensis (Wight) Mull.Arg. Locally called "mootipazham" moderately sized evergreen tree of the Euphorbiaceae family, traditionally the leaves are used by tribals in Kerala against inflammation by drinking the water which is boiled with the parts of fruits, bark and leaves. The leaf paste are applied on swellings and also used for other anti-inflammatory purposes. The fruits of *B. courtallensis* are consumed as seasonal fruits and their leaves are cooked like side dishes and consumed with rice or rice soup by the kani tribes of Kerala. The selection of *Baccaurea courtallensis* is based on the tribal claim to assess the anti-oxidant and anti-inflammatory properties. However till date no scientific validation of these properties have been reported. Hence the present study was carried out to scientifically evaluate the anti-inflammatory and anti-oxidant potential of *Baccaurea courtallensis* (Wight) Mull.Arg.

2. Materials and methods
2.1. Plant material
The leaves of *Baccaure*a *courtallensis* (Wight) Mull.Arg. locally known as Mootipazham were collected from the campus of Iqbal College, Peringammala. The plant is endemic to southern, western ghats and throughout distributed in the districts of Kerala. The specimens of the plant were deposited in the herbarium of the institute with accession No: TBGT-30457 dt 01-03-2016.

2.2. Extraction
Collected leaves were shade dried then again kept in oven for drying. This is then powdered using a mechanical grinder. 40g of powdered plant material was extracted by cold extraction method using solvent ethanol. Obtained crude extracts were evaporated and removed excess ethanol by using a rotary evaporator.

2.3. Experimental Animals
Wistar rats (170 to 275 gm) and Swiss albino mice, (35 to 45 gm), of either sex, were obtained from the animal house of JNTBGRI, Palode. They were grouped and housed in poly acrylic cages (three animals per cage) and maintained under standard laboratory conditions (24 - 28° C, relative humidity 60-70% and 12 hours' dark light cycles). They were fed commercially rat feed (Lipton India Ltd, Mumbai, India) and boiled water (ad libitum). All animals were carried out according to NIH guidelines, after getting the approval of the Institutes Animal Ethics committee.

2.4. Acute toxicity study
Six groups of 2 mice were administered 50, 150, 450, 1400, 2500 and 5000 mg/kg of BC extract respectively, maintaining appropriate controls. All the animals were observed continuously for the first 3 h then 1 h intermittently up to 24 h for behavioral changes like convulsions, hyperactivity, sedation, grooming, loss of righting reflex, epilation, respiratory rate, food and water intake, state of faecal pellets, and mortality. The animals were observed for post treatment toxic symptoms daily for 14 days after treatment.

2.5. Anti-inflammatory activity-Carrageenan induced paw oedema
The anti – inflammatory activity was studied in groups of three rats. Oedema was induced according to the method of Neha *et al*., (2013) [16]. Briefly, 0.1ml of 1% carrageenan was injected into the right hind paw, under the planatar aponeurosis (carrageenan controls). A similar volume of 0.5% Tween80 was injected into the left hind paw of the animals in one group (vehicle controls). The hind paw volume of the vehicle controls and the carrageenan controls and the drug treatment groups was measured before carrageenan injection plethysmographically and the time course of edema formation was followed for 3 hours. In a separate group of animals, indomethacin (10 mg/kg) was administered orally 30 min before carrageenan injection. The volume of increase of the inflamed paw was estimated by substracting the volume of the control hind paw. The anti-inflammatory activity of the plant extract was esteemed as the degree of oedema inhibition. Antioxidant studies DPPH based free radical scavenging activity method The free radical scavenging activities of Baccaurea courtallensis extract (BCE) was carried out using DPPH based free radical scavenging activity method (Blois *et al*., 1958) [7]. To 2ml of 0.2 Mm DPPH, 0.2 ml of BCE in methanol at varying concentrations (25, 50, 100, 150, 200 µg/ml) was added. Simultaneously a control was prepared without adding the extract. The reaction mixture was mixed well and incubated for 20 minutes in the dark at 28 C. Then the scavenging activity of each concentration of BCE was determined by measuring the absorbance (abs) of the decolorized solution at 515 nm using a spectrophotometer against the control solution.

2.6. Antioxidant studies DPPH based free radical scavenging activity method
The free radical scavenging activities of *Baccuarea courtallensis* extract (BCE) was carried out using DPPH based free radical scavenging activity method (Blois *et al*., 1958) [7]. To 2ml of 0.2 Mm DPPH, 0.2 ml of BCE in methanol at varying concentrations (25, 50, 100, 150, 200 µg/ml) was added. Simultaneously a control was prepared without adding the extract. The reaction mixture was mixed well and incubated for 20 minutes in the dark at 28 C. Then the scavenging activity of each concentration of BCE was determined by measuring the absorbance (abs) of the decolorized solution at 515 nm using a spectrophotometer against the control solution. Total antioxidant activity using phosphomolybdenum method The total antioxidant capacity of *B. courtallensis* extract (BCE) was obtained by phosphomolybdenum method (Prieto *et al*., 1999) [17]. 0.2 ml of BCE solution (200 µg/ml) in the respective solvent was mixed with 2 ml of reagent solution (6 M sulphuric acid, 28 mM sodium phosphate,4 mM ammonium molybdate). The reaction mixture was incubated at 95 c for 90 min. The blank solution contained 2 ml of reagent solution and the approximate volume of same solvent used for the sample. The absorbance of the solution was measured 695 nm against blank. The antioxidant capacity of BCE was evaluated as equivalents of ascorbic acid.

2.7. Phytochemical analysis
Preliminary phytochemical tests were carried on the extracts to detect the various constituents present in them (H. Wagner, 1993; J.B Harborne, 1998; Gahan, 1984).

2.7.1. Detection of Alkaloids
To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test-tube. A reddish brown precipitate confirms the presence of alkaloids.

2.7.2. Detection of Flavonoids Shinoda's test
The extract (50 mg) was dissolved in 5ml of alcohol and few fragments of magnesium ribbon and concentrated HCL acid drop wise added. Presence of flavonol glycosides was inferred by the development of pink to crimson colour.

2.7.3. Detection of Phenols
The methanol extract is treated with magnesium turnings followed by concentrated HCL acid which is added in drops. The appearance pink scarlet /intense red/green to blue colour indicates the presence of phenols.

2.7.4. Detection of Tannins
About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (10mg in 10ml) was added and observed for brownish green or a blue black colorization.

2.7.5. Detection of Coumarins
To the test solution add a few ml of alcoholic sodium hydroxide solution. The appearance of intense yellow color on addition of con. HCL acid indicates its presence of Coumarin.

2.7.6. Detection of Steroids
Fehling's test 1ml of filtrate was treated with each 1ml of fehling's solution A and B and boiled in a water bath. A reddish precipitate was obtained which shows the presence of sugar.

2.7.7. Detection of oils
A small quantity of extract was pressed between two filter paper oil stain on the paper indicates the presence of fixed oils.

2.7.9. Detection of Saponins
50 mg of extract is diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for

15 minutes. The formation of 2 cm layer of foam indicates the presence of saponin.

2.7.10. Detection of Anthraquinones
0.5g of the extract was boiled with 10 ml of Conc. sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

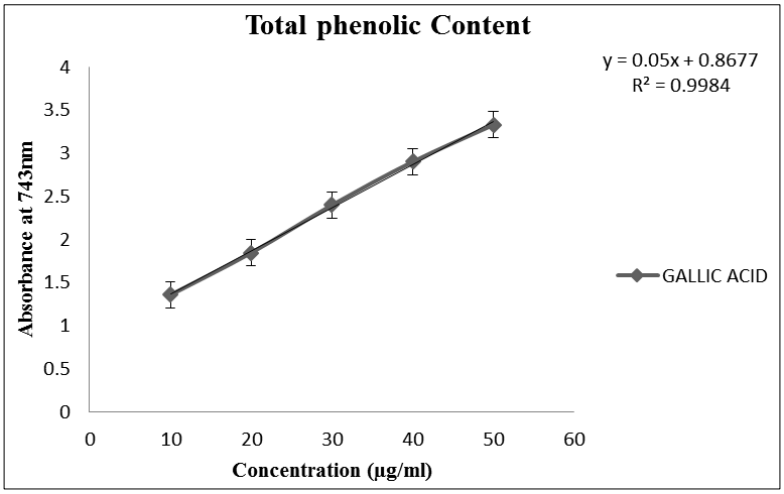
2.7.11. Detection of Aminoacids
To 2ml of extract add 2drops of ninhydrin (10 mg of ninhydrin in 200 ml of acetone). Purple colour indicates the presence of amino acids.

2.7.12. Detection of Proteins
Biuret test: An aliquot of 2ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1ml of ethanol (95%) was added, followed by excess of KOH pellets. Pink colour in the ethanolic layer indicates the proteins.

2.8. Estimation of total phenolic content
Total phenolic content (TPC) of BCE extract was determined according to the method described by Lachman *et al*. (2000). 0.5 ml of each extract, 2.5 ml Folin-Ciocalteu reagent, 2 ml of 7.5% (w/v) sodium carbonate (Na2CO3) were mixed. The mixture was incubated at room temperature for 30 min. The absorbance was read using UV-Vis spectrophotometer at 743 nm. Each analysis was performed in triplicates and the values were expressed in mean ± standard deviation. The results were expressed as mg GAE (gallic acid equivalents)/g ethanolic extract.

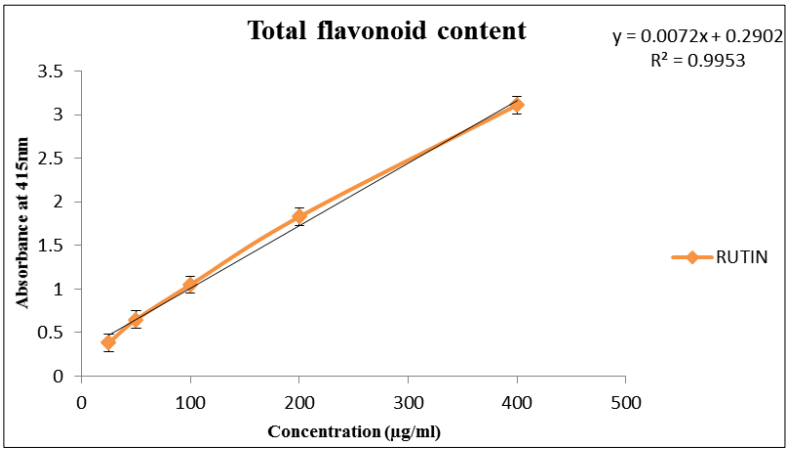
2.9. Estimation of total flavonoid content
The total flavonoid content (TFC) of BCE extract was determined according to the Aluminum chloride colorimetric method described by Chang *et al*. (2002). The plant extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% Aluminum chloride hexahydrate (AlCl3.6H2O), 0.1 ml of 1 M potassium acetate (CH3CO2K), and 2.8 ml of distilled water. After incubation at room temperature for 30 min. the absorbance of the solution was measured at 415 nm. Each analysis was performed in triplicates and the values were expressed in mean ± standard deviation. The results were expressed as mg RE (rutin equivalents)/g ethanolic extracts.

3. Results
3.1. Quantification of total Phenolic content in *Baccaurea courtallensis* Leaves ethanolic extract
From the standard calibration curve, total phenolic content was found to be 131mg GAE/g ethanolic extract (Graph 1).



Graph 1: Quantification of total phenolic content in ethanolic extract of *Baccuarea courtallensis* Leaves.

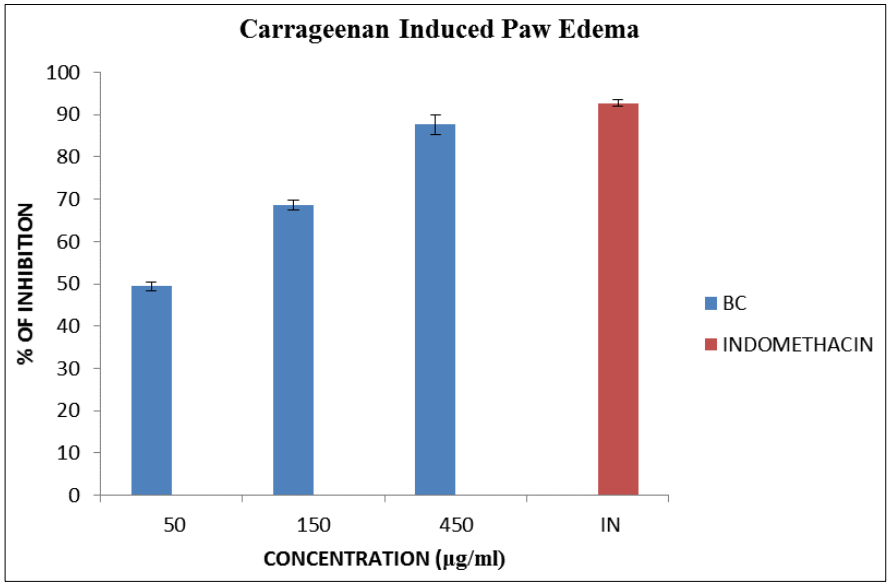
3.2. Quantification of total flavonoid content in *Baccaurea courtallensis* Leaves ethanolic extract
From the standard calibration curve, total flavonoid content was found to be 72.2 mg Rutin Equivalent/g of dry water extract (graph 2).



Graph 2: Quantification of total flavonoid content in ethanolic extract of *Baccuarea courtallensis* (Bc). Leaves

3.3. Effect of *Baccaurea courtallensis* (BC) ethanolic leaf extract on carrageenan induced rat paw oedema

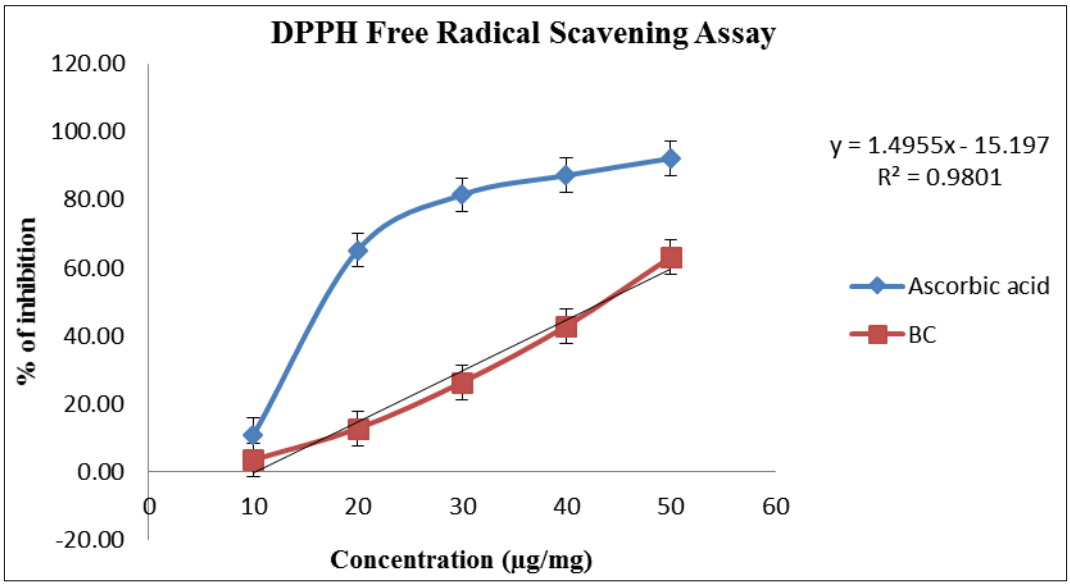
Baccaurea courtallensis (BC) leaf ethanolic extract at the doses of 150 and 450 mg/kg significantly inhibited the carrageen induced rat paw edema in rats. At 150 mg/kg dose, there was 68.18% inhibition and at 450 mg/kg dose 86.36% inhibition, at three hours after carrageenan injection. Indomethacin (10mg/kg) produced 92.72% inhibition of edema formation (graph 3).



Graph 3: Effect of ethanolic extract of *Baccuarea courtallensis* (BC) leaves on carrageenan induced rat paw edema:

3.4. Effect of *Baccaurea courtallensis* (BC) leaf ethanolic extract in DPPH radical scavenging activity

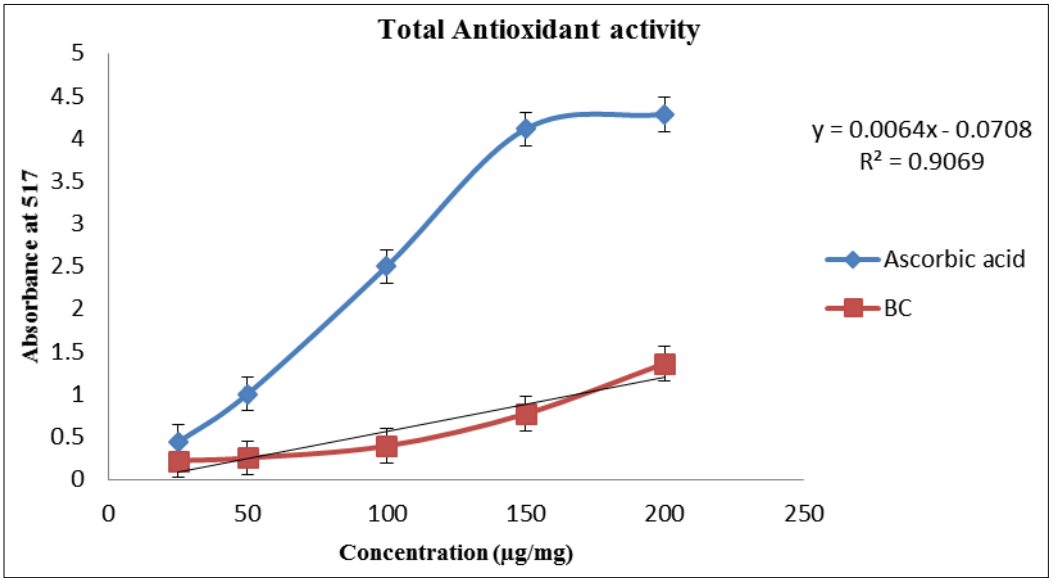
The ethanolic leaf extract of *Baccaurea courtallensis (*BC*)* at the dose of 50µg/ml exhibited the significant free radical scavenging activity (86.34 %) of DPPH (1, 1 – diphenyl -2-picrylhydrazyl). The BC extract exhibited an IC_{50} at a concentration of 43.60 µg/ml (graph 4).



Graph 4: Effect of ethanolic extract of *Baccuarea courtallensis* (BC) leaves in DPPH radical scavenging activity:

3.5. Measurement of total antioxidant property of *Baccuarea courtallensis* (BCE) by phosphomolydenum method

The total antioxidant activity of BCE at a concentration of 200µg/ml was found to be equivalent to the activity produces by 238 µg/ml of ascorbic acid (graph 5).



Graph 5: Effect of ethanolic extract of *Baccuarea courtallensis* BC) leaves in total antioxidant activity by phosphomolybdenum method:

3.6. Phytochemical analysis shows (table 1)

Table 1: phytochemical analysis shows the presence and absence of following phytochemicals.

Compounds	Ethanolic extract of *B.courtallensis* (leaves)
Alkaloids	++
Flavonoids	+
Phenols	++
Tannins	+
Carbohydrates	+
Saponins	-
Anthraquinones	+
Coumarin glycosides	+
Proteins	-
Amino acids	-
Steroids	+
Fixed oil	-

+ indicates presence of compound and - indicates absence of compound.

Acute toxicity study

Result of acute toxicity study showed that there was no mortality or any significant change in the behavior of the mice after the administration of *Baccaurea courtallensis*(BC) in graded doses which showed nontoxic effect of the drug for 24 hours (table 2)

Table 2: Acute toxicity studies shows the following observations.

Parameters	Cage side observation
Mortality	Nil
Exophthalmic	Nil
Subcutaneous swellings	Nil
Writhing	Nil
Muscle spasm	Nil
Sedation	Nil
Skin	Normal
Salivation	Nil
Ptosis	Nil
Lacrimation	Nil

Discussion

Phytochemical bioactive compounds are secondary plant products that have been related to the medicinal properties of diverse plants. Preliminary phytochemical analysis on BC extract showed the presence of alkaloids, flavonoids, phenols, tannins, carbohydrates, anthraquinones, coumarin glycosides and steroids. These phytochemicals are exhibit medicinal as well as physiological activities (Sofowra, 1993). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function (Singh *et al*., 2007) [18]. The methanol extracts of *Baccaurea courtallensis* were subjected to preliminary phytochemical analysis and they showed the presence of steroids, coumarins, tannins, flavanoids, phenols, quinones and volatile oils (Abhishek *et al*., 2012).

DPPH radical is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Studies reported that consumption of foods high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, since they act as antioxidants (kaur *et al*., 2002). The total phenolic content (TPC) of BC were quantified based on the linear equation obtained from Gallic acid standard calibration curve as 131mg GAE/g.

The total antioxidant activity of ethanolic extract of *B. courtallensis* was estimated from their ability to reduce Phosphate/Mo (VI) complex to Phosphate/Mo (V) by the extract and subsequent formation of green phosphate/ Mo (V) complex at acid pH.

According to Yan, Asmah and Oktay *et al*., (2003), phenolics present in edible plants have received considerable attention because of their potential antioxidant activity. Phenolics compounds undergo a complex redox reaction with the phosphotungstic and phosphor-molybdic acid present in the Folin-Ciocalteu reagent. The report suggests that 98% of the plant antioxidant activity results from the activity of phenolic compounds. The inhibitory concentration (IC50) value for ethanolic extract of *Baccaurea courtallensis* was found to be 43.60 µg/ml, indicating that it contains high level of antioxidants. In addition, there was a positive correlation occurred (R^2 = 0.980) between total phenolic content and antioxidant activity in this study. Also it can be concluded that the antioxidant activity of the plant is not limited to phenolics. Activity may come from the presence of other

antioxidant secondary metabolites such as flavonoids, volatile oils and carotinoids. The finding in this study was also agreement with study by Velioglu *et al*. (1998) and Standley *et al*., (2001) whereby they demonstrated a linear relationship between antioxidant capacity and total phenolic in plant products.

Flavonoids are polyphenols subclasses which are widely distributed in the plant kingdom and are characterized by two or more aromatic rings, each bearing at least one aromatic hydroxyl and connect with a pyrin. Flavonoids belong to a group of natural substances occurring normally in the diet that exhibit a variety of beneficial effects on health. The anti-inflammatory properties of flavonoids have been studied recently, in order to establish and characterize their potential utility as therapeutic agents in the treatment of inflammatory diseases (Ana Garcia *et al*., 2009). The estimation of flavonoid content of *Baccaurea courtallensis* was done using Aluminium chloride Calorimetric method described by Chang *et al*., 2002. Total flavonoid contents was calculated using the standard curve of rutin ($y = 0.006x + 0.290$; $R^2 = 0.9975$) and was expressed as rutin equivalents (RE) per gram of the plant extract.

No mortality was recorded for the oral administration of graded doses of ethanolic extract of *Baccaurea courtallensis* for all the doses after 24 hours of administration to the animal. The anti-inflammatory activity was studied using carrageenan induced rat paw edema test at different doses (50,150 and 450 mg/kg body weight) of the ethanol extract of BC leaves. Carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation. Carrageenan-induced oedema involves the synthesis or release of mediators at the injured site. The reduction in the paw oedema after pretreatment with BC may be due to the suppression of increased vascular permeability caused by the extract. The anti-inflammatory property of BC may be due to the inhibition of proinflammatory mediators, free radical scavenging, or membrane stabilizing effects. According to (Gomes *et al*., 2008: Shale *et al*., 1999) flavonoids (or bioflavonoids) are naturally occurring compounds in vascular plants and have been considered to possess anti-inflammatory properties, both *in vitro* and *in vivo*. The anti-inflammatory activity of the ethanolic extract of BC may also be proved due to the presence of flavonoids in a significant amount (72.2 mg rutin equivalent per g of dry extract).

Conclusion

Since the plant extract significantly reduced the formation of oedema induced by Carrageenan, the leaves of *B. courtallensis* exhibited acute anti-inflammatory activity. The potential of the extract as an acute anti-inflammatory agent may be due to the presence of phytoconstituents such as flavonoid and tannins. Presence of phenolics is the main phytochemicals responsible for the high antioxidant activity. The plant extract is nontoxic up to the dose of 1400mg/kg; it showed that the plant might be safe for use. Therefore, it can be concluded that the ethanolic extract of *B. courtallensis* leaves possess anti-inflammatory activity and justify its use as a traditional folk remedy for inflammation, pain etc. However, a more extensive and detailed study of *B. courtallensis* is needed to determine the exact mechanism of action. Detailed Phytochemical studies are warranted against this for the phytocompounds present on it. Hence *B. courtallensisis* is a good candidate in the future for drug development in the treatment of inflammatory disorders and also as a good antioxidant.

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