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Inhibition of cyclooxygenase activity by standardized extract of *Givotia rottleriformis* Griff. ex Wight bark

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A standardized ethanol extract of *Givotia rottleriformis* Griff. ex Wight bark was tested *in vitro* for anti-inflammatory activity on key pro-inflammatory enzymes, cyclooxygenase (COX-1 and COX-2) and membrane stabilizing potential. The extract was standardized in terms of the presence of flavonoids by HPLC, total phenolics by Folin-Ciocalteu method, flavonoid content using $AlCl_3$ method, and free radical scavenging activity using inhibition of hydroxyl, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and nitric oxide radical. The anti-inflammatory effect was screened using cyclooxygenase inhibition and membrane stabilizing potential. In the HPLC analysis, 4 flavonoids were identified by comparing with the calibration curve derived from the standard rutin, quercetin, kaempferol, and luteolin. The total phenolic content and flavonoid contents were found to be 13.80 and 5.7 % w/w, respectively. Significant hydroxyl, DPPH, and nitric oxide radical scavenging activity was observed with IC_{50} values of 230, 220, and 180 µg/mL, respectively. The ethanol extract significantly protected the rat erythrocyte membrane against lysis induced by hypotonic solution. The ability of the extract to inhibit cyclooxygenase enzymes (COX-1 and COX-2) was determined by calculating percent inhibition of PGE_2 production measured by enzyme immunoassay. The extract inhibited both enzymes with IC_{50} value of 45 and 37 µg/mL, respectively. The anti-inflammatory activity of *G. rottleriformis* bark could be at least in part due to free radical-scavenging activity and cyclooxygenase enzyme inhibition.

Keywords: *Givotia rottleriformis* Griff. ex Wight, Polyphenol, Anti-inflammatory, COX-1/COX-2.
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Introduction

Plant extracts are believed to contain potential bioactive component that can strongly inhibit the expression of cyclooxygenase (COX). Therefore, there is a continuous need to search for new drugs from natural products with anti-inflammatory properties having minimum side effects. Modulation of the enzymes' activities implies that the inflammation process can be modified.

COX, which exists in at least two isoforms, catalyzes the first key steps in the synthesis of all the prostaglandins (PGs) by converting arachidonic acid (AA) into PGH2. Thus, COX is a bifunctional enzyme exhibiting both cyclooxygenase (from AA to PGG2) and peroxidase (from PGG2 to PGH2) activities. COX-1 is constitutively expressed as a housekeeping enzyme in nearly all the tissues and mediates physiological responses (such as cytoprotection of the stomach and platelet aggregation). On the other hand, COX-2 is expressed by cells that are involved in inflammation and has emerged as the isoform primarily responsible for the synthesis of prostanoids involved in acute and chronic inflammatory states of pathological processes[1]. Prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs) is also associated with severe side effects such as gastrointestinal hemorrhage due to COX-1 inhibition[2]. The new COX-2 selective drugs do not seem to be free of risk either, since several COX-2 inhibitors have been found to cause cardiovascular problems[3].

The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species (ROS) from activated neutrophils and macrophages. The released ROS leads to tissue injury

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by damaging macromolecules and lipid peroxidation of membrane. Thus, free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation[4]. The use of plants is still a large source of natural antioxidants, which serve as a lead for the development of novel drugs[5].

Givotia rottleriformis Griff. ex Wight, a moderately sized tree is commercially valuable in building Catamarans. The plant belongs to the family Euphorbiaceae and is distributed in limited areas of the forests of Tamil Nadu, Andhra Pradesh, Karnataka, West Bengal, and coastal Sri Lanka. The bark and seeds of the tree are used in indigenous medicine for the treatment of inflammatory diseases such as rheumatism, psoriasis, and dandruff[6]. The present study evaluated the anti-inflammatory potential of standardized ethanol extract of *G. rottleriformis* using a COX inhibition assay in order to validate and establish scientific evidence for its ethnobotanical uses.

Materials and Methods

Plant material

The plants material was collected from the forest of Attur, Salem district, Tamil Nadu. It was identified by Dr P Jayaraman, Director, Plant Anatomy Research Centre, Tambaram, Chennai and a voucher specimen (No. PARC/2011/2140) was deposited for further references.

Extraction

About 500 g of the *G. rottleriformis* bark powder was extracted using a soxhlet apparatus with ethanol (70 % v/v) for 18 h. The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator). The total extract (10 g) thus obtained was subjected to chemical test for identification of various plant constituents.

Phytochemical screening

Concentrated ethanol (70 % v/v) extract was screened for the presence of various phytoconstituents using standard protocol[7].

HPTLC analysis

To support preliminary chemical analysis, ethanolic extract was subjected to HPTLC studies. Chromatograph was performed on 10 x 10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel $60F_{254}$ (E. Merck Ltd, Darmstadt, Germany) stored in a dessicator, application was done by Hamilton microsyringe (Switzerland), mounted on a Linomat V applicator. Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 25±20 ^{o}C with benzene: methanol: ammonia as a mobile phase in a camag chamber previously saturated for 30 min. After development, the plate was dried at 60 ^{o}C in an oven for 5 min. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with win CATS Software and the chromatograms were recorded[8].

Fingerprint analysis by HPLC

Method of Boligon *et al*.[9] was used for the qualitative and quantitative analysis of the sample. The HPLC consisting of a pump (model Jasco PU2080, intelligent HPLC pump) with injecting facility programmed at 20 µL capacity per injection was used. The detector consisted of a UV-Vis (Jasco UV 2075 model) operating at a wavelength of 270 nm. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system. The column was Thermo ODS Hypersil C18 (250 x 4.6 mm, 5 µm) in isocratic mode. The separation was achieved using a mobile phase of methanol, water, and phosphoric acid (100: 100: 1, v/v/v) at a flow-rate of 1.5 mL/min. The mobile phase was filtered through 0.45 µm nylon filter prior to use.

Sample preparation

Powdered drug of *G. rottleriformis* bark was weighed and transferred into a 250 mL flask fitted with a reflux condenser. About 78 mL of extraction solvent (Alcohol: water: hydrochloric acid, 50:20:8) was added, refluxed on a hot water bath for 135 min, cooled at room temperature and transferred into a 100 mL volumetric flask. About 20 mL of methanol was added into the 250 mL flask, sonicated for 30 min and filtered. The filtrate was transferred into a 100 mL volumetric flask and the residue was washed on the filter with methanol. The washing was collected in the same 100 mL volumetric flask and diluted to volume. The same method was followed to prepare the standard stock solutions of the flavonoids. Identification was based on retention times and on-line spectral data in comparison with standard flavonoids. Quantification is performed by establishing calibration curves for each determined compound, using the standards.

Determination of total phenolic content

The content of total phenolics in the powdered *G. rottleriformis* bark was determined by using Folin-Ciocalteu reagent. About 1 g of the powder was extracted in an ultrasonic wave bath with 80 mL of

aqueous ethanol solution (70 % v/v) for 2 h. After cooling, the volume of the solution was adjusted to 100 mL. The final solution was centrifuged prior to the colorimetric determination. Tannic acid standards (10-110 mg/mL) were dissolved in 100 mL of aqueous ethanol solution (70 % v/v) respectively. About 10 mL of Folin-Ciocalteu reagent was added to 1 mL of the extract solution and 1 mL of standard solution. After reacting for 3 min, 10 mL of 35 % sodium carbonate solution was added and the test solution was diluted to 100 mL with water and mixed. After 45 minutes, an aliquot was centrifuged for 5 min. The absorption coefficient for the supernatant was measured at 745 nm. The total phenolic content of the extract was calculated using the mean regression coefficient from the standard[10].

Determination of total flavonoids

Total flavonoid content in dried aerial parts of the plant was estimated by spectrometric method using Perkin-Elmer UV-Vis spectrometer Lambda 16 (Germany). Dried and powdered bark (10 g) was extracted by continuously mixing in 100 mL of 70 % ethanol for 24 h at room temperature. After filtration, ethanol was evaporated until only water remained. Water phase was subsequently extracted with ethyl acetate. The extract was dried over anhydrous sodium sulphate, filtered, and concentrated under vacuum up to a concentration of 1 g/mL of extract. They were further diluted with ethyl acetate to obtain 0.01 g solutions. About 10 mL of the solution was transferred into a 25 mL volumetric flask, to which 1 mL of 2 % $AlCl_3$ was added. The solution was filled to volume with methanol-acetic acid and kept aside for 30 min. The absorbance was measured at 390 nm against the same solution without $AlCl_3$ being blank. Rutin was used to construct the calibration curve in the concentration range 1.0-10.0 µg/mL[11].

Experimental animals

Healthy male Wistar rats (120-170 g) obtained from the animal house of Vels University, Tamil Nadu were used for the study. Animals were housed in polypropylene cages and were left for 7 days to acclimatize to room maintained under controlled condition (12 h light–12 h dark cycle at 22±2 ^oC) on standard pellet diet and water ***ad libitum***. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA with prior approval of the Institutional Animal Ethics Committee (IAEC/52/2012).

***In-vitro* anti-inflammatory activity**

Membrane stabilizing activity

Erythrocyte suspension was prepared by obtaining whole blood with heparinized syringes from the rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged each time for 10 min at 3000 g[12]. Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte hemolysis. The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the test sample (50, 100, and 200 µg/mL) or indomethacin (0.1 mg/mL). The control sample consisted of 0.5 mL of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g. Absorbance of the supernatant was measured at 540 nm. The percentage inhibition of hemolysis or membrane stabilization was calculated by the following formula:

$$\text{Membrane stabilization} = 100 \times (OD_1\text{-}OD_2/OD_1)$$

Where, OD_1 is optical density of hypotonic-buffered saline solution alone and OD_2 is optical density of test sample in hypotonic solution.

Cyclooxygenase inhibitory activity

In vitro COX-2 inhibiting activities of the compounds have been evaluated using 'COX (ovine) inhibitor screening assay' kit with 96-well plates. Both ovine COX-1 and COX-2 enzymes were included. This screening assay directly measures PGF2 produced by $SnCl_2$ reduction of COX-derived PGH2. COX-1, COX-2, initial activity tubes were prepared by taking 950 µL of reaction buffer, 10 µL of heme and 10 µL of COX-1 and COX-2 enzymes in respective tubes. Similarly, COX-1, COX-2 inhibitor tubes were prepared by adding 20 µL of inhibitor (ethanol extract) in each tube in addition to the above ingredients. The inhibitory assays were performed in the presence of extracts at different concentrations (10-50 µg/mL). The background tubes correspond to inactivated COX-1 and COX-2 enzymes obtained after keeping the tubes containing enzymes in boiling water for 3 min along with vehicle control. Reactions were initiated by adding 10 µL of arachidonic acid in each tube and quenching with 50 µL of 1 M HCl. PGH2 thus formed was reduced to PGF2α by adding 100 µL $SnCl_2$. The prostaglandin produced in each well

was quantified using broadly specific prostaglandin antiserum that binds with major prostaglandins and reading the 96-well plate at 405 nm. The wells of the 96-well plate showed low absorption at 405 nm indicating the low level of prostaglandins in those wells and hence, less activity of the enzyme. Therefore, the COX inhibitory activities of the compounds could be quantified from the absorption values of different wells of the 96-well plate[13]. Indomethacin (selective COX-1 inhibitor) and celecoxib (selective COX-2 inhibitor) were used as positive controls in the study. The test extract concentration causing 50 % inhibition of PGE_2 release (IC_{50}) was calculated from the concentration-inhibition response curve by regression analysis.

Antioxidant activity

Hydroxyl radical scavenging activity

The assay was performed by adding 0.1 mL EDTA, 0.01 mL ferric chloride, 0.1 mL hydrogen peroxide, 0.36 mL deoxyribose, 1 mL ethanol extract (50-400 µg/mL), 0.33 mL phosphate buffer (50 mM, pH 7.4), 0.1 mL ascorbic acid in sequence and incubating at 37 ^{o}C for 1 h. A portion (1.0 mL) of the incubated mixture was mixed with 1.0 mL of 10 % Trichloroacetic acid and 1.0 mL of 0.5 % thiobarbituric acid to develop the pink chromogen, which was measured at 532 nm[1].

DPPH radical scavenging activity

About 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of the different concentration (50-400 µg/mL) of ethanol extract and control (without the test compound, but with an equivalent amount of methanol) in different test tubes. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer[14].

Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH 7.4) was incubated with different concentrations of ethanolic extract (50-400 µg/mL) dissolved in phosphate buffer saline (0.025 M, pH 7.4) and the tubes were incubated at 25 ^{o}C for 5 h. Control experiments without the test compounds but equivalent amounts of buffer were conducted in an identical manner. After 5 h, 0.5 mL of incubation solution was removed and diluted with 0.5 mL of Griess reagent (1 % sulphanilamide, 2 % O-phosphoric acid, and 0.1 % naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with N-(1-naphthyl)ethylenediamine was read at 546 nm[14]. All the tests were performed in 6 replicates and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples by the following formula:

$$\text{Percentage inhibition (\%)} = \frac{(\text{Absorbance of control-Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

Level of significance of all the parameters was expressed as the arithmetic mean ± SEM and was analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's t test. *P* value less than 0.05 ($P < 0.05$) was the critical criterion for statistical significance. IC_{50} was calculated from the concentration-inhibition response curve by regression analysis

Results and Discussion

About 500 g of the *G. rottleriformis* bark powder was extracted using a soxhlet apparatus with ethanol (70 % v/v) and the yield of ethanol (70 % v/v) extract was about 6.4 % w/w.

Phytochemical screening

Preliminary phytochemical screening of ethanol (70 % v/v) extract showed positive results for alkaloids, steroids, flavonoids, tannins, terpenoids, and saponins.

HPTLC analysis

The ethanolic extract was further subjected to HPTLC for the conformation of the active constituents. The ethanolic extract showed nine resolutions of spot with the solvent system benzene: methanol: ammonia (9:0.5:0.5). Out of 9 components, the component with R_f values 0.04, 0.16, 0.30, 0.53, and 0.77 were found to be more predominant, as the percentage area was more with 15.06, 20.55, 22.04, and 15.08 %, respectively. The remaining components were found to be very less in quantity, as the percent area for all the spots were less than 9.0 %. The R_f values are given in Fig. 1.

Fingerprint analysis of HPLC

The HPLC chromatograms of the bark of *G. rottleriformis* showed 7 components (Fig. 2). The main difference was in the peak eluted at 3.96, 11.52, 16.68, and 30.76 min, respectively. In the present

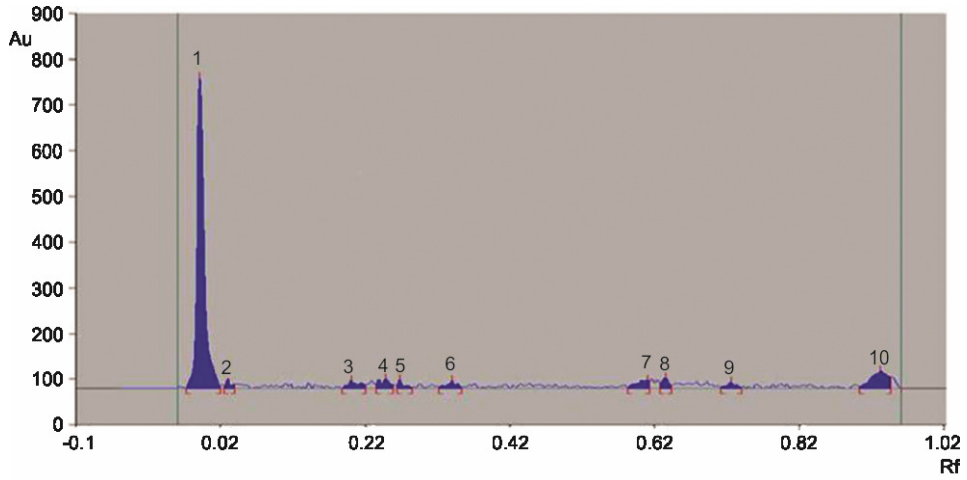
investigation, 4 flavonoids were quantified at 254 nm using peak area by comparison to a calibration curve derived from the standard, rutin (0.215 mg/g), quercetin (1.36 mg/g), kaempferol (6.36 mg/g), and luteolin (8.64 mg/g) (Fig. 3). The results show that the bark of *G. rottleriformis* is a rich source of the important biologically active flavonoids, rutin, quercetin, kaempferol, and luteolin. The described HPLC procedure could be useful for the qualitative and quantitative analysis of flavonoids in plant materials.

Estimation of phytoconstituents

The total phenolic and total flavonoid content were observed to be 13.80 and 5.7 % w/w, respectively.

Anti-oxidant activity

Several concentrations ranging from 50-400 µg/mL of the ethanolic extract were tested for their antioxidant activity in different *in vitro* models. It was observed that the free radicals scavenging property of the test was found to be concentration dependent in all the models (Table 1).



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	0.8	-0.01	678.5	83.02	0.02	7.1	6414.1	78.90	unknown*
2	0.03	6.2	0.03	18.7	2.28	0.04	9.3	138.2	1.70	unknown*
3	0.19	0.5	0.20	15.7	1.92	0.22	7.6	257.4	3.17	unknown*
4	0.23	8.7	0.25	19.3	2.37	0.26	8.6	287.3	3.53	unknown*
5	0.26	6.1	0.27	17.5	2.14	0.29	2.2	144.3	1.77	unknown*
6	0.32	3.5	0.34	15.8	1.94	0.35	3.2	213.0	2.62	unknown*
7	0.58	2.3	0.61	16.5	2.02	0.61	14.6	303.6	3.73	unknown*
8	0.63	12.4	0.64	21.5	2.63	0.64	6.9	199.1	2.45	unknown*
9	0.71	3.43	0.73	13.8	1.68	0.74	2.4	172.9	2.13	unknown*

Fig. 1—Peak densitogram display of ethanolic extract of the *Givotia rottleriformis* bark

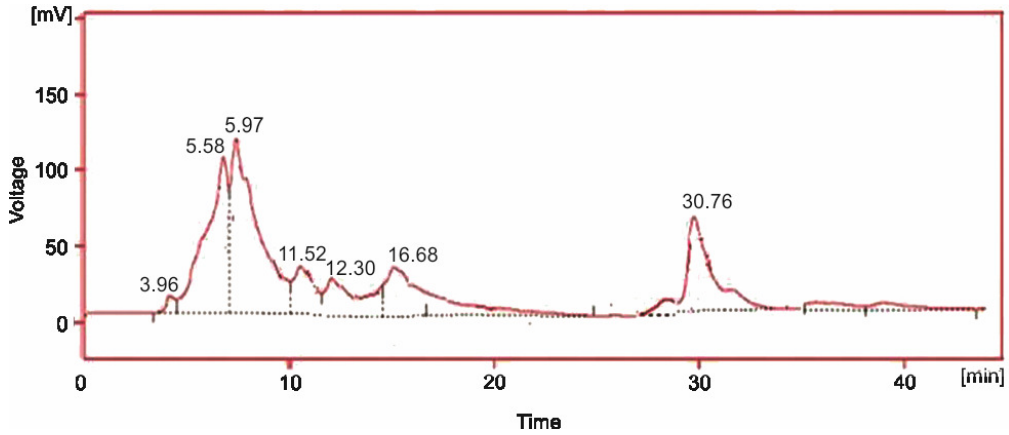


Fig. 2—HPLC Profile of ethanol extract of *Givotia rottleriformis* bark

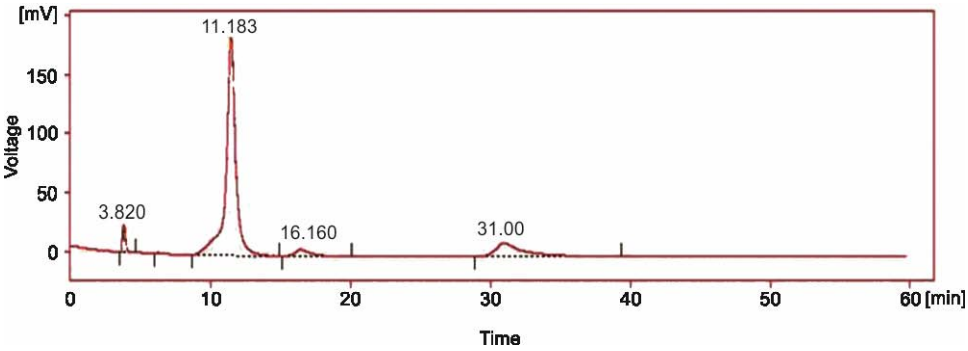


Fig. 3—HPLC of reference standards: 1-Rutin 2-Quercetin, 3-Kaempferol, and 4-Luteolin

Table 1—Free radical scavenging activity of ethanol extract of *Givotia rottleriformis* bark

Drugs	Concentration (µg/mL)	Hydroxyl radical inhibition (%)	DPPH radical inhibition (%)	Nitric oxide inhibition (%)
Ethanol extract	50	17.18±1.24	14.05±2.48	13.57±0.70
	100	23.62±2.12	22.83±3.26	27.40±2.26
	200	43.24±1.60	48.50±2.82**	56.62±1.35**
	300	65.12±1.36**	68.67±0.90**	74.31±1.79**
	400	87.72±3.27**	92.89±0.68**	88.26±1.28**
	IC_{50}	230 µg/mL	220 µg/mL	180 µg/mL
Ascorbic acid	5	14.31±1.20	12.38±1.80	10.81±1.72
	10	30.46±3.09	20.68±1.28	18.68±0.62
	20	59.68±1.38**	41.36±1.82	28.26±2.31
	30	88.25±1.49**	62.84±1.82**	44.36±1.28**
	40	94.60±2.30**	80.82±2.18**	70.10±2.16**
	IC_{50}	18 µg/mL	24 µg/mL	35 µg/mL

Values are Mean±SEM of 6 parallel measurements. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's 't' test (n=6). **P < 0.01, when compared against control.

Hydroxyl radical activity

The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids, and proteins. The effect of ethanol extract of *G. rottleriformis* on the inhibition of free radical-mediated deoxyribose damage was assessed by means of iron (II)-dependent DNA damage assay, which showed significant results. The IC_{50} value was 230 µg/mL.

Inhibition of DPPH radical

DPPH assay is considered a valid and an easy to evaluate scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as in other radical assays. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compound and plant extract to act as free radical scavengers. The potential decrease in the concentration of DPPH radical due to the scavenging ability of ethanol extract showed significant free radical scavenging activity of about 92.89±1.35 % at higher doses with the IC_{50} value being 220 µg/mL.

Nitric oxide scavenging activity

Nitric oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases. Nitric oxide is a very unstable species under aerobic conditions. It reacts with superoxide to produce stable product nitrate and nitrite through intermediates NO_2, N_2O_4, and N_3O_4[14]. It is estimated by using Griess reagent and in the presence of test compound, which is a scavenger that the amount of nitrous acid will decrease. In the present study, nitrite produced by the incubation of solutions of sodium nitro prusside in standard phosphate saline buffer at 25 ^oC was reduced by the ethanol extract. Significant scavenging activity was observed for the extract with an IC_{50} value of 180 µg/mL. This may be due to the antioxidant principles of flavonoid, which compete with oxygen to react with nitric oxide, leading to reduced production of nitric oxide.

The best-described property of almost every group of flavonoids is their capacity to acts as antioxidants. Quercetin, kaempferol, morin, myricetin, and rutin, by acting as antioxidants exhibited beneficial effects such as anti-inflammatory, antiallergic, antiviral, as well as anticancer activity. Significant hydroxyl, DPPH, and nitric oxide radical scavenging activity was observed for the extract, which may be due to the presence of flavonoid like rutin, luteolin, kaempferol, and quercetin.

In-Vitro anti-inflammatory activity

Effect on erythrocyte membrane stability

The ethanol extract (50-200 µg/mL) significantly protect the rat erythrocyte membrane against lysis induced by hypotonic solution. The compounds with membrane-stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators and offer significant protection of cell membrane against injurious substance, thereby exhibit anti-inflammatory property[15]. When the human RBC is subjected to hypotonic stress, the hemoglobin release from RBC is prevented by anti-inflammatory drugs because of the membrane stabilization. The ethanol extract exhibited 36 % inhibition of RBC hemolysis at 100 µg/mL as compared with 43 % produced by indomethacin at 100 µg/mL. The ethanol extract demonstrated significant membrane stabilizing property contributing a significant role to its anti-inflammatory activity.

Inhibition of COX enzymatic activity

Developing novel, effective, and safe anti-inflammatory agents is a major research area in finding alternatives to NSAIDs. Anti-inflammatory agents possessing selective COX-2 inhibition and showing negligible or no effect on COX-1 activity are more appreciated as safe drugs due to their minimum gastrointestinal side effects. Natural products, especially medicinal plants and drug discovery have remained a very successful combination for the inventorization of new therapeutic agents. PGE2 level increases markedly and provokes inflammation and pain in pathological events due to the activation of COX enzymes. Hence, the ethanolic extract of *G. rottleriformis* (10-15 µg/mL) bark was tested for its ability to inhibit COX-1 and COX-2. The extract exhibited a significant anti-inflammatory activity *in vitro* by inhibiting both COX-1 (56.4 %) and COX-2 (68.9 %), respectively at higher concentration of 50 µg/mL (Fig. 4). The IC_{50} values of ethanol extract obtained for COX-1 was 45 µg/mL and for COX-2 was 37 µg/mL. Standard inhibitors were indomethacin for COX-1 and celecoxib for COX-2.

Phytochemicals like flavonoids, terpenoids, alkaloids, and saponins have been described to possess significant anti-inflammatory activity. Several studies have proved that naturally occurring coumarins and flavonoids act as dual inhibitors of cyclooxygenase and 5-lipoxygenase activities[16]. The Indian spice turmeric, (*Curcuma longa* L.) containing curcumin (and synthetic analogs) has established reputation as an anti-inflammatory agent by inhibiting COX-1 and COX-2[17]. Flavonoids inhibit biosynthesis of prostaglandins (the end products of the COX and lipoxygenase pathways), which acts as a secondary messengers and are involved in various immunologic responses. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory disorders[18].

Flavonoids are well studied class of polyphenols as COX-2 inhibitors. Kaempferol and quercetin exhibited anti-inflammatory activities by inhibiting inducible nitric oxide synthase (iNOS) and COX-1/COX-2 protein levels in cultured human umbilical vein endothelial cells[19]. Luteolin and galangin, well known flavonoid molecules were studied as first dietary polyphenols as inhibitors of arachidonic acid peroxidation[20]. After this chrysin and luteolin were considered potent anti-inflammatory agents as they

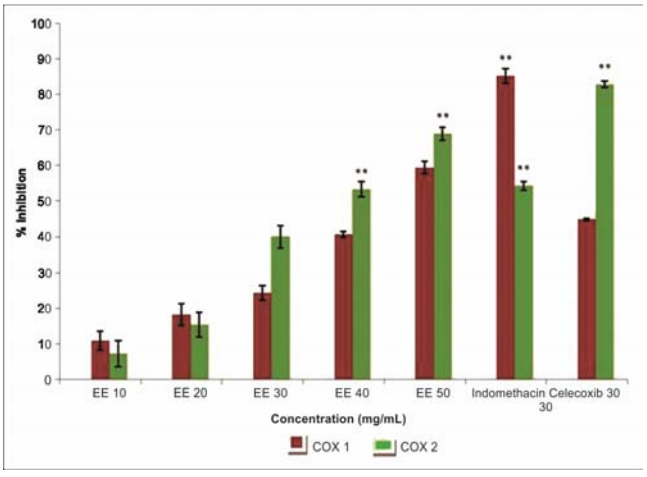


Fig. 4—*In vitro* percentage inhibition for COX-1 and COX-2 enzymes by ethanol extract of *Givotia rottleriformis* bark. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6), $^{**}P < 0.01$, when compared against control.

effectively suppressed COX-1/COX-2 activity[21]. There are some reports on the beneficial role of some dietary polyphenols in inhibiting arachidonic acid peroxidation and possessing COX-1/COX-2 inhibitory effects[22]. The present investigation showed that *G. rottleriformis* bark inhibited both COX-1 and COX-2 indicating their non-selective anti-inflammatory ability which may be contributed by the presence of significant levels of flavonoids like rutin, quercetin, kaempferol, and luteolin.

Conclusion

The ability of the ethanol extract to inhibit cyclooxygenase pathway of arachidonate metabolism has been suggested to contribute to anti-inflammatory activity. Further work on *in vivo* anti-inflammatory evaluation of the extract in an animal model is needed to confirm the therapeutic potential. The results of the present study provided a scientific support for the use of *G. rottleriformis* bark establishing a pharmacological evidence for the folklore claim of the drug to be used in the chronic inflammatory diseases in ethnomedicine.

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