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PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTI-INFLAMMATORY POTENTIAL OF EXTRACT OF *CHLOROXYLON SWIETENIA*

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ABSTRACT

Chloroxylon swietenia is a traditional medicine hence the present work is conducted to evaluate antimicrobial and anti-inflammatory activity. Hydroalcoholic extract of *Chloroxylon swietenia* leaf was assessed for its antimicrobial and anti-inflammatory activity and phytochemical screening. Total phenolic content and total flavonoids content was assessed. The antimicrobial efficacy was determined using paper disc method. Zones of inhibition and phytochemical composition of extract was determined. In vitro anti-inflammatory activity was evaluated using by using Carrageenan-induced paw edema model. The phytochemical screening showed that the hydroalcoholic extract of *Chloroxylon swietenia*, the flavonoids, carbohydrates, phenol, diterpenes and saponins were present in leaf extract. The antimicrobial activities of hydroalcoholic extract of *Chloroxylon swietenia* gave different zones of inhibition on the organisms tested at different concentrations. The extract showed potent anti-inflammatory activity, presented also highly significant statistic values (*p<0.05, **p<0.01, ***p< 0.001). Findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial and anti-inflammatory agent from *Chloroxylon swietenia* leaves.

Keywords: Orodispersible tablets, Clopidogrel bisulfate, Solid dispersions, Solubility, Dissolution rate.

INTRODUCTION

The wide usage of commercial antimicrobial drugs leads to side effects, and most notably, development of drug resistance in the majority of the pathogenic microbes is an another major problem. To minimize the synthetic antimicrobial drugs and to reduce side effects, screening of plants with antimicrobial properties is growing day-by-day. It is well known that several medicinal plants have been used to treat microbial infections from 1000 years [1, 2]. A number of studies reported the antimicrobial properties of plants [3-9]. In addition to medicinal value, there is an increasing interest toward natural antioxidants

present in plants. Antioxidant molecules have the capability to quench free radicals and remediate the effects caused by reactive oxygen species and have roles in the prevention of degenerative diseases which caused by oxidative stress [10]. Medicinal plants are rich sources of antioxidant molecules such as flavonoids, triterpenoids, tannins, coumarins, quinones, vitamins, and polyphenolic compounds and it was reported by several studies [11-13].

Chloroxylon swietenia is the sole species in the genus Chloroxylon, belonging to the family Rutaceae and is commonly known as bherul in Sanskrit and satinwood in English. *Chloroxylon swietenia* provides a decorative timber, used for furniture, pattern making, interior trim, cabinet work, flooring, boxes, crates, interior joinery, carvings, toys, musical instruments and luxury goods [14]. *Chloroxylon swietenia* is considered as a medicinal plant having several medicinal uses. Leaves paste is used to treat wounds, snakebites and rheumatism. Bark extract is used to treat chest pain and asthma. In friction it is used to treat bruises and painful joints. To treat impotence root bark in milk is drunk in Sri Lanka [15]. Leaves are used to treat worm infected wounds, fungal infected skin and also to treat inflammation, rheumatism. The plant root and bark possess astringent property [16]. The leaves, stem bark and seeds using the hydroalcoholic solvent have not been exposed yet and need to be evaluated which may yield a novel phytoconstituent for the prevention of infections caused by clinical pathogens. Therefore, the present study was conducted on preliminary phytochemical analysis, antimicrobial and anti-inflammatory potential of *Chloroxylon swietenia*.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade and were used without additional purification.

Collection of plant material

Leaves of *Chloroxylon swietenia* were collected from rural area of Bhopal (M.P), India in the months of November, 2019.

Extraction by soxhletion method

45.7 gm dried powdered leaves of *Chloroxylon swietenia* was extracted with hydroalcoholic solvent (ethanol: water; 80:20) by soxhletion method using soxhlet's apparatus for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [17, 18].

Total phenols determination

The amount of total phenolic contents of extract was determined by the spectrophotometric method of Kim et al., [19] with slight modification. A diluted plant extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample. The solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23 °C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The total phenolic content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried sample.

Total flavonoids determination

The total flavonoids assay was conducted according to Katasani Damodar [20]. Total flavonoids content was determined by using aluminium chloride colorimetric method. Aqueous extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 0 to 150 µg/ml in methanol. The total flavonoids content was expressed as milligrams of Quercetin equivalents per gram of dried sample.

Antimicrobial activity

Diameter of zone of inhibition was determined using the paper disc diffusion method as described by Lai et al. [21] and Adedapo et al. [22]. A swab of the bacteria suspension containing 1×10^8 cfu/ml was spread on to Petri plates containing nutrient agar media. Each extracts were dissolved in ethanol to final concentration of 10 mg/ml. Sterile filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37°C for 24 h. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

***In vivo* anti-inflammatory activity**

Selection of animals

In the present investigation the Swiss albino rats of either sex, weighing between 150-250 g were used. The animals were procured from College of Veterinary Sciences and Animal Husbandry, Mhow, (M.P.), India. Animals were allowed to acclimatize for two weeks before commencing the study and maintained under standard laboratory conditions (25±2ºC temperature, 45-65% relative humidity and 12 h light and

12 h dark cycle). The animals were fed with standard laboratory animal feed and water *ad libitum* throughout the study. The animal experimental protocol was duly approved by the Institution Animal Ethical Committee.

Acute oral toxicity

Acute oral toxicity was performed according to Organization for Economic Co-operation and Development (OECD) guideline No. 420. Swiss albino rats were fasted overnight, accessing water *ad libitum* were used in this study. The extract was administered orally at a dose of 2000 mg/kg body weight and the animals were observed for mortality or any abnormal behavior for first 24 h, then for next 14 days. Further behavioral responses, neurological responses as well as autonomic responses were observed.

Evaluation of *in vivo* anti-inflammatory activity by using Carrageenan-induced paw edema model. Carrageenan-induced paw edema was evaluated according to the method described by Winter et al., [23]. Rats were divided into four groups; each group consisting of six animals. Paw edema was induced by injecting 0.1 ml of 1% *w/v* carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat.

Table 1: Anti-inflammatory activity by using Carrageenan-induced paw edema model

Group	Treatment
Group I (Negative control)	Carrageenan (0.1 ml of 1% *w/v*)
Group II (Standard control)	Carrageenan (0.1 ml of 1% *w/v*) + Diclofenac sodium (10 mg/kg, p.o.) as standard reference
Group III (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (100 mg/kg, p.o.) of *Chloroxylon swietenia* leaves
Group IV (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (200 mg/kg, p.o.) of *Chloroxylon swietenia* leaves

The paw thickness was measured before injecting the carrageenan and after 60, 120, 180 min. using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group.

The percentage (%) inhibition of edema is calculated using the formula:

$$\% \text{ Inhibition} = \frac{To - Tt}{To} \times 100$$

Where T_t is the thickness of paw of rats given test extract at corresponding time and To is the paw thickness of rats of control group at the same time.

Data analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

RESULTS AND DISCUSSION

The phytochemical screening showed that the hydroalcoholic extract of *Chloroxylon swietenia*, the flavonoids, carbohydrates, phenol, diterpenes and saponins were present in leaf extract. The alkaloids were absent in the extract.

Table 2: Phytochemical screening of leaves extract of *Chloroxylon swietenia*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Hager's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenolics**	
	$Fecl_3$	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Fehling's test	+ve
7.	**Saponins**	
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve

-ve= Negative, +ve= Positive

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X-0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R^2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 3: Total phenolic and total flavonoid content of extract of *Chloroxylon swietenia*

S. No.	Extract	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	**Hydroalcoholic extract**	0.543	0.965

The total phenolic content found in the hydroalcoholic extract of *Chloroxylon swietenia* was 0.543 mg/100gm (Table 3). The total flavonoid content was 0.965 mg/100 mg in the hydroalcoholic extract, (Table 3). Phenols and flavonoids seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The flavonoids from plant extracts have been found to possess antioxidants, antimicrobial and anti inflammatory properties in various studies [24, 25].

The antimicrobial activities of hydroalcoholic extract of *Chloroxylon swietenia* gave different zones of inhibition on the organisms tested at different concentrations. The obtained results revealed that hydroalcoholic extract of *Chloroxylon swietenia* ranged from 8±0.76 mm (25mg/ml hydroalcoholic extract concentration), 11±0.86 mm (50 mg/ml hydroalcoholic extract concentration), and 14±0.57 mm (100 mg/ml hydroalcoholic extract concentration) for *Staphylococcus aureus* and 8±0.94 mm (25mg/ml hydroalcoholic extract concentration), 13±0.57 mm (50 mg/ml hydroalcoholic extract concentration), and 16±0.5mm (100 mg/ml hydroalcoholic extract concentration) for *Pseudomonas aeruginosa* respectively. The differential sensitivity of Gram positive and Gram negative bacteria to plant extracts may be explained by the morphological differences between the microorganisms [26].

Table 4: Antimicrobial activity of hydroalcoholic extract of *Chloroxylon swietenia* against selected microbes

S. No.	Name of microbes	Zone of inhibition Hydroalcoholic extract		
		25mg/ml	50mg/ml	100mg/ml
1.	*Staphylococcus aureus*	8±0.76	11±0.86	14±0.57
2.	*Pseudomonas aeruginosa*	8±0.94	13±0.57	16±0.5

No adverse changes and mortality were observed in animals, which orally received hydoralcoholic extract (2000 mg/kg) of *Chloroxylon swietenia*. This indicates that 2000 mg/kg is maximum safe dose. So $1/20^{th}$ and $1/10^{th}$ *i.e.* 200 and 400 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* anti-inflammatory effects.

Carrageenan-induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory agents. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve. The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component. Table 5 shows the effect of hydro-alcoholic extract of *Chloroxylon swietenia* leaves and diclofenac sodium (standard drug) as compared to carrageenan control at different hours in carrageenan-induced paw edema model using vernier caliper. Hydro-alcoholic extract administered at a dose of 100mg/kg p.o prevented carrageenan-induced paw edema with a percentage inhibition of 23.64%, 35.73%, 41.24%, and 59.60% at 1, 2, 3, and 4 hours, respectively, while 29.84%, 44.76%, 59.78%, and 74.78% at a dose of 200mg/kg p.o. at 1, 2, 3.and 4 hour, respectively. Diclofenac sodium at a dose of 10 mg/kg p.o. prevented carrageenan-induced paw edema with a percentage inhibition of 55.40%, 59.81%, 68.32%, and 76.40% at 1, 2, 3.and 4 hour, respectively.

Table 5: *In vivo* anti-inflammatory activity by using Carrageenan-induced paw edema model

Group	Dose of extract (mg/kg, p.o.)	Change in paw thickness (mm)±SD (% inhibition)			
		1 hr	**2hr**	**3hr**	**4hr**
Group I (Negative control)	Carrageenan (0.1 ml of 1% *w/v*)	3.48±0.18	5.59±0.23	6.32±0.24	7.15±0.20
Group II (Standard control)	Carrageenan (0.1 ml of 1% *w/v*) + Diclofenac sodium (10 mg/kg, p.o.)	1.46±0.18** (55.40%)	1.72±0.12** (59.81%)	1.82±0.14*** (68.32%)	1.47±0.18*** (76.40%)
Group III (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (100 mg/kg, p.o.)	2.50±0.11* (23.64%)	2.75±0.12* (35.73%)	3.37±0.11** (41.24%)	2.51±0.17*** (59.60%)
Group IV (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (200 mg/kg, p.o.)	2.30±0.07* (29.84%)	2.36±0.19** (44.76%)	2.31±0.17*** (59.78%)	1.57±0.09*** (74.78%)

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with toxicant control group (one-way ANOVA followed by Dunnett's test).Values in parentheses indicate percent inhibition activity (H), calculated as 100 x (value of negative control – value of treatment) / value of negative control

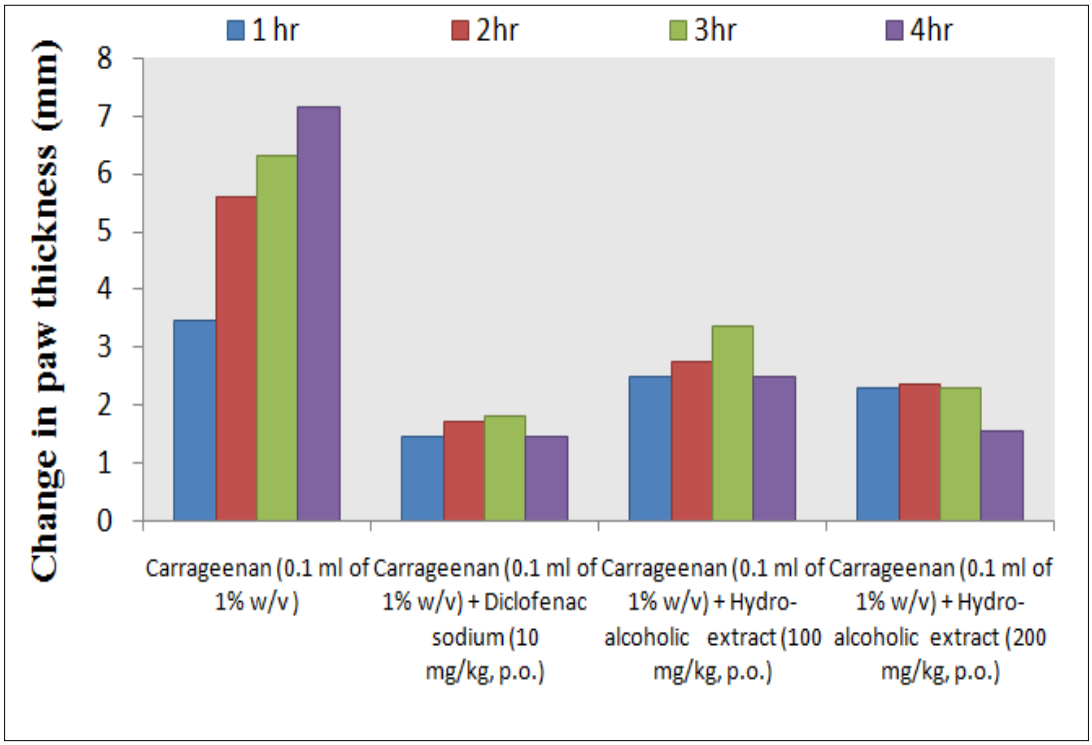

Figure 1: Percentage protection against inflammation induced by Carrageenan-induced paw edema on treatment with hydro-alcoholic extract of *Chloroxylon swietenia* leaves

The extract showed potentanti-inflammatory activity, presented also highly significant statistic values (*p<0.05, **p<0.01, ***p< 0.001). The ability of the extract to inhibit carrageenan induced paw edema is suggestive of its anti-inflammatory potential. Anti-inflammatory effects have been observed in flavonoids as well as tannins. Flavonoids such as quercetin are known to be effective in reducing acute inflammation. Certain flavonoids possess potent inhibitory activity against a variety of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A_2, phosphodiesterases and others. The anti-inflammatory effect of the extract may be due to the presence in the extract of flavonoids, tannins *etc.* either singly or in combination [27]. This may be due to the presence of active phytoconstituents *i.e.* flavonoids, and due to their effect on the prostaglandins pathway.

Conclusions

The present investigation has shown that the hydro-alcoholic extract of *Chloroxylon swietenia* leaves have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial and anti-inflammatory agent from *Chloroxylon swietenia* leaves. From the above study it can be suggested that the hydro-alcoholic extract of

Chloroxylon swietenia leaves promising anti-inflammatory activity. This effect may be beneficial for the management of pain.

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