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Anthelmintic activity of *Alangium salviifolium* bark

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ABSTRACT

Alangium salviifolium (AS) is a novel medicinal plant used for the treatment of various diseases including helminthiasis by the traditional healers of Chhattisgarh. So an attempt has been taken to explore it scientifically. Preliminary phytochemical investigation reveals that there is presence of flavonoids, Saponins, phenols, bitter principles and steroids. Three different concentrations (50, 100 and 150 mg/ml) of crude extract of hexane, ethyl acetate, chloroform and methanol were tested against earthworms (*Pheretima posthuma*), which involved the evaluation of paralysis and death period of the worm. Methanol and chloroform extracts exhibited significant anthelmintic activity at highest concentration of 150 mg/mL. Piperzine citrate was selected as standard compound and DMF as control. The anthelmintic activity of Methanol and chloroform of bark of AS has therefore been evaluated for the first time.

Key words: *Alangium salviifolium*; helminthiasis; *Pheretima posthuma*; Piperzine citrate and Paralysis

INTRODUCTION

Helminthiasis is a macroparasitic disease of humans and animals caused due to parasitic worms such as pinworm, roundworm, or tapeworm. These worms some times live in the gastrointestinal tract, may also burrow into the liver, lymphatic system, or other organs [1]. The clinical picture of consists of skin infection, skin nodules, and ocular lesions. The skin lesions include oedema, papules, scab-like eruptions and altered pigmentation. World Health Organisation (WHO) has identified helminthiasis as the world's second leading cause of permanent and long-term disability after leprosy [2]. In India it is a major public health problem. This type of disease was recorded in India as early as 6th century B.C. by the famous Indian physician, Susruta in his book 'Susruta Samhita' [3]. Number of synthetic drugs like mebendazole, albendazole, piperazine, pyrantel, and mebendazole used to control and prevent from the infection, used as broad spectrum anthelmintic drug [4]. Long term used of this synthetic medicine associated with various adverse effects like tolerance, resiststance, nausea, vomiting drowsiness, dizziness, and abdominal pain [5]. Therefore, search for new drugs is essential from natural source. Since the time man first discovered the plant kingdom as a rich and convenient source of his food, he returned to this kingdom repeatedly and found remedies for illness too. As a result, several knowledge-bases of how to treat or prevent illness and diseases using plants were generated [6]. Chhattisgarh, the Herbal state in India, is situated in Deccan biogeographical area. The forests of the state fall under two major forest types, i.e., Tropical Moist Deciduous forest and the Tropical Dry Deciduous forest. The state of Chhattisgarh is endowed with about 22 varied forest sub-types existing in the state. Most of the medicinal valuable species are in the category of on-Nationalized Non-Wood Forest Produces are the one on which the state does not have any monopolistic control. The rich and unique biological diversity comprises of 911 genera and 196 families. The state is significantly rich in endemism with

respect to many plants having medicinal importance [7, 8]. Many traditional healers and herbalists in the Chhattisgarh region of India have been treating helminthiasis patients for many years using various medicinal plant species [9]. So a intensive plant survey have been performed to explore plants effective against helminthiasis used by the traditional healers of Chhattisgarh.

Alangium salvifolium (L.f.) Wang (Dhera in hindi) (AS) is a small tree, with more or less spinescent branches. Leaves 7.6-15.2 cm long, narrowly oblong or ovate-lanceolate, glabrous. Flowers few in axillary fascicles. Fruits small, nearly globular, purplish-red when ripe, crowned by persistent calyx-lobes. The genus consists of 24 species of small trees, shrubs and lianas, and is native to western Africa, Madagascar, southern and eastern Asia (China, Malaysia, Indonesia, and the Philippines), tropical Australia, the western Pacific Ocean islands, and New Caledonia. Most of the species are native to tropical and subtropical regions of east and southeast Asia [10]. Five of the species extend well outside of this area. *Alangium platanifolium* extends from east Asia into Russia. *Alangium chinense* (sensu lato) extends from south east Asia to Africa. *Alangium salviifolium* is the most widespread species, ranging from Africa to Australia, Fiji, and New Caledonia. *Alangium villosum* occurs from southeast Asia to Australia and the western Pacific Islands. *Alangium grisolleoides* is endemic to Madagascar and gives the genus a disjunct distribution. As per the traditional knowledge Plant pacifies vitiated pitta. It is anti-hypertensive, antidote for several poisons especially for rabies. Roots are useful for external application in case of rheumatism and inflammation. Fruits are used in treatment of hemorrhages. Root bark is emetic, febrifuge, purgative, anthelmintic, diaphoretic, antipyretic; useful in fever and piles. It is also used in leprosy, syphilitic and other skin diseases. Leaves are useful as poultice in rheumatic pains. Fruits are laxative, expectorant, carminative, anthelmintic, alexiteric; useful in inflammation, burning of the body, spermatorrhoea, gleet, acute fever and lumbago. Based on this concept and as per my search for natural source as anthelmintics, a suitable plan has been designed to explore methanolic extract of this plant scientifically as anthelmintic agent.

MATERIALS AND METHODS

Collection and Extraction of Plant material:

Bark of *Alangium salviifolium* was collected in the month of August from the tribal belt of Chhattisgarh. The bark were converted in to coarse powdered through size reduction. The powdered drug was then extracted with hexane, ethyl acetate, chloroform and methanol successively by soxhlet apparatus. All the extracts were concentrated to a semisolid mass by vacuum evaporation and stored in freeze for further study.

Preliminary Phytochemical analysis:

Methanolic extract of AS was subjected to qualitative chemical screening for the identification of the various major classes of active chemical constituents. Test for flavonoids: 2ml of the extract was filtered and 1ml of the filtrate was mixed with dilute NaOH, golden yellow precipitate confirmed the presence of flavonoids. Test for phenols: 2ml of the extract was mixed with 3ml 5% ferric chloride and five drops of potassium ferricyanide, dark green precipitate confirmed the presence of phenols. Test for steroids/saponins: 1 g of the extract was mixed with 10 ml of warm distilled water, frothing persistent indicated the presence of saponins. An additional test was performed by Liebermann–Burchard test. To 100mg of extract, 2ml of acetic anhydride was added; the mixture was thoroughly stirred, heated for 2 min on a water bath and allowed to stand at room temperature. When 2ml of sulfuric acid was gently added to 0.7 ml of a supernatant acetic anhydride layer, the upper layer gave a blue to green colour confirming the presence of steroidal saponins [11].

Evaluation of Anthelmintic activity:

Adult Indian earthworm (*Pheretima posthuma*) is having anatomical and physiological resemblance with the intestinal round worm of human beings. So In-vitro anthelmintic activity was performed in *Pheretima posthuma*, collected from moist soil of the field and washed with normal saline solution. *Pheretima posthuma* of 2-4 cm in length and 0.1-0.2 cm in width were selected for all the experimental parameters. Eighty four worms were selected for study in fourteen groups (twelve test, one standard group, and one control) having six in each group. Three different concentrations (50, 100 and 150 mg/ml) of crude extract of hexane, ethyl acetate, chloroform and methanol were prepared in DMF. Each group earthworms were taken in petriplates. Different concentration of extracts in DMF was pour into the petriplates. Observations were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis in min was noted when no movement of the worm. Time of death in min was recorded after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm

water (50°C). Piperazine citrate (PC; 10 mg/mL) [5] was used as standard. Treatment with normal saline served as control.

RESULTS AND DISCUSSION

In recent years, there has been an increased inclination towards the herbal medicines due to the trend towards the natural sources and a healthy life style. Moreover, the complexity, side effects and costly treatment associated with the allopathic drugs have caused both the healthcare practitioners and the majority of world populations to turn towards alternative therapies, more likely towards the herbal medicines [12]. In this present paper I have evaluated a Indian medicinal plant *Alangium salviifolium* for its anthelmintic potential, which is being used by the traditional healers.

Preliminary Phytochemical study of this plant collected from Chhattisgarh reveals that there is presence of flavonoids, Saponins, phenols, bitter principles and steroids. Further work by various authors suggests that Leaves, roots and seeds contain the alkaloids, ankorine, marckidine, marckine, tubulosine, alangicine, cephaeline, psychotrine, etc.; steroids and triterpenoids. Plant (root, leaves and fruit) also yield monoterpenoid lactam, alangiside, loganic acid, venoterpine, dl-salsoline and isocephaeline. Leaves also contain alkaloids, deoxytubulosine, alangimarckine, dehydroprotoemetine etc [13]. Root bark contains the alkaloids, emetine, cephaeline, psychotrine, tubulosine, isotubulosine alangium A, alangium B, marckidine, marckine, and alangine, and also myricyl alcohol, stigmaterol and β -sitosterol. Root also contains de-Me-psychotrine and a new alkaloid, alangicin. Stem bark contains the alkaloids, alangine, akharkantine, akoline and lamarkine. Seven tetrahydroisoquinoline monoterpene glucosides have been isolated from the fruits. Seed alkaloids include emetine, cephaeline, N-methylcephaeline and psychotrine. They also contain betuline, betulinaldehyde, lipoal, betulinic acid and β -sitosterol. Stigmasta- 5, 22, 25-trien-3 β -ol, myristic acid, E-cis- fused neohopane derivatives, alangidiol and its isomer; N- benzoyl-L-Ph-alaninol, and 3 unidentified triterpenoids also isolated from the plant. Which may be responsible for its therapeutic activity.

Different extracts of AS in the concentration range of (50g/ml, 100mg/ml and 150mg/ml) were evaluated for anthelmintic activity, shown in table-1. One-way ANOVA study among various groups reveals that there was significant difference in time taken for paralysis ($P < 0.05$) of earthworm. Post-hoc test revealed that methanolic extract (150 mg/mL), and chloroform extract (100 mg/mL) groups were not significantly different compared to PC in time taken for paralysis of earthworm, indicating equivalence in potency. Except this all the treated groups showed significant difference in time of paralysis compared to PC (10 mg/ml). Furthermore, statistical analysis by One-way ANOVA showed that there was significant difference in time taken for death ($P < 0.05$) of Indian earthworm among groups. The post-hoc test indicated that the time taken for death of earthworm as similar to that of the effect observed in time taken for paralysis of earthworm, indicating equivalent potency while compared to PC. Present Paper reveals that the methanolic extract (150 mg/ml) and Chloroform extract (150 mg/ml) have equivalent potency compared to PC (10 mg/ml) in time taken for both paralysis and death of earthworm, shown in table-1.

Table1. Anthelmintic activity of different extracts of bark of AS on *Pheretima posthuma*

Extracts	Concentration (mg/mL)	Time taken for Paralysis (Min)	Time taken for death (Min)
Hexane	50	88±0.4	108±0.5
	100	65±0.3	78±0.4
	150	59±0.3	72±0.3
Ethyl acetate	50	96±0.4	121±0.2
	100	56±0.9	102±0.9
	150	49±0.8	93±0.7
Chloroform	50	78±0.3	96±0.5
	100	59±0.6	60±0.6
	150	42±0.5	53±0.4
Methanol	50	70±0.4	99±0.3
	100	46±0.3	79±0.1
	150	32±0.2	61±0.2
PC	10	26±0.3	53±0.4
Control	-	-	-

The mode of action of PC reveals that it acts by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization so excitability decreases, which leads to muscle relaxation and flaccid paralysis [14] thus, it may be a possible mode of action of AS. Therefore, future study can be under taken for standardization of

each extracts and isolation of phytoconstituents in each extracts to establish the mechanism of action. Furthermore, the pharmacological studies for anthelmintic activity should be undertaken in other parasites to mimic the exact human helminthiasis.

CONCLUSION

The methanolic and chloroform extract of root of *C. swietenia* showed anthelmintic activity on *Pheretima posthuma*. Therefore, future study can be under taken for standardization of each extracts and isolation of phytoconstituents in each extracts to establish the mechanism of action. Further, the pharmacological studies for anthelmintic activity should be undertaken in other parasites to mimic the exact human helminthiasis.

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