ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2013; 4(1): 235-239

ORIGINAL RESEARCH ARTICLE

Antimicrobial and Antihelminthic Activities of Various Extracts of Leaves and Stems of *Abutilon indicum* (Linn.)

Pusapati Madan Ranjit[1]*, Y. Ankamma Chowdary[1] , Harika Krapa[1], Sindhu Nanduri[1], Himabindhu Badapati[1],, Kola Phani Kumar[2], Priyanka Bommadevara[1], Madhuri Kasala[1]

[1]NRI College of Pharmacy, Pothavarappadu (V), Agiripalli Mandal, Krishna (Dist.), A.P, India
[2]ANU College of Pharmaceutical Sciences, Nagarjuna Nagar, Nagarjuna University, Guntur (Dist.), A.P, India

Received 28 Oct 2012; Revised 07 Feb 2013; Accepted 18 Feb 2013

ABSTRACT

Our present study carried out the antimicrobial and antihelminthic activity of leaves and stems of the plant *Abutilon indicum* (Linn.) Sweet (Malvaceae).Different parts of the plant are used for treatment of various ailments in ethnomedicine. We are carried out the antimicrobial activity chloroform extract prepared form leaves using agar-well diffusion method against both gram positive and gram negative microorganisms and antihelminthic activity was performed by using alcoholic extract of stems, ethyl acetate and carbon tetra chloride fraction of aqueous extracts from the leaves of plant against *Pheretima posthuma*.

Key words: Antimicrobial activity, Antihelminthic activity, ethnomedicine, *Abutilon indicum*, *Pheretima posthuma.*

INTRODUCTION

Abutilon indicum (Linn.) Sweet (Malvaceae) is a shrub distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in variety of ailments in traditional system of medicine such as Ayurveda and Siddha. The roots are useful in treating uterine hemorrhagic discharges. Leaves are useful in treatment toothache, lumbago, piles and all kinds of inflammation. Bark is used as anthelmentic and diuretic [1]. In addition it also showed antispasmodic, cardiac depressant, estrogenic, antifungal and hepatoprotective properties [2-5]. Dried whole plant was used as laxative and demulcent [6], febrifuge, anthelmintic, diuretic, anti–inflammatory, especially in uterine discharges, piles, lumbago [7] anti inflammatory, immuno stimulant, piles and gonorrhea treatment. Roots and bark are used as aphrodisiac, anti diabetic, nervine tonic, and diuretic. Seeds also used as aphrodisiac, in treatment of urinary disorders [8]. The whole plant is reported to have analgesic [9], hypoglycemic [10], hepato protective [11], hyperlipidemic activity [12]. The various leaves extract was reported to contain alkaloids, flavonoids, sterols, triterpenoids, and glycosides [13-15]. The other phytochemical constituents present in this plant are include fatty acids, abutilin A, flavonoids, quercetin, glycosides, alkaloids, steroids, terpenoids, saponins, sesquiterpenes, lactones, gallic acid, β-sitosterol, geranoil, caryophylline and phenolic compounds[16 -18]. The aim of the present research is, to determine the preliminary phytochemical constituents, antimicrobial and antihelmenthtic activity of various extracts of the leaves and stems of *Abutilon indicum.*

MATERIALS AND METHODS

Collection of plant material:

Abutilon indicum (linn.) leaves and stem are collected in the month of July and August from the area Pothavarappadu (V), Agiripalli (M), Krishna District, Vijayawada. The collected parts of the plant materials were authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati (DRM/NRI/15/08/2011/AC2). Herbarium specimen was deposited in the Department of Pharmacognosy, NRI College of Pharmacy. (Specimen no.NRI/COL/P.COG/AILS).

***Corresponding Author:** Pusapati Madan Ranjit**, Email:** madanranjit@gmail.com

Preparation of extracts:

The various parts of the plant dried under the shed and subjected to grinding. The powered material was subjected to extraction in soxhlet extractor by using solvents. The leaves are extracted with chloroform (Yield of extract 7.15g) and water. The aqueous extract was further fractioned by using ethyl acetate (Yield of extract 4.23g) and carbon tetrachloride (Yield of extract 1.4g). Likewise, they were stems powered and extracted with alcohol (Yield of extract 2.7g).

Preliminary phytochemical investigation:

The qualitative chemical tests of various extracts of plant were carried out using standard procedures [19]. Preliminary phytochemical investigation shows the presence of alkaloids, saponins, carbohydrates flavonoids, phenolic compounds, steroids and tannins.

ANTIBACTERIAL ACTIVITY:

Test organisms:

Antibacterial activity was performed by using chloroform extract of the leaves against selected NCIM (National Collection of Industrial Microorganisms) type bacterial stains. These are four Gram positive micro-organisms (*Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2063, *Bacillus pumilis* NCIM 2327, and *Micrococcus lutes* NCIM 2871) and three Gram Negative microorganism (*Escherichia coli* NCIM 2067, *Pseudomonas aeruginosa* NCIM 2037, and *Proteus vulgaris* NCIM, 2027).

Preparation of inoculums:

The *in vitro* screening of antibacterial activity was carried out using Cylinder-plate assay method. For antibacterial activity, the inoculums or microbial suspension is prepared according to the procedure given in the I.P (Indian pharmacoepia-2010).The test organism (one loop full) were seeded to the nutrient agar (HIMEDIA) at temperature between 40° and 50° and immediately pour the inoculated medium into the Petri plate (8 Inch) to give a depth of 3 to 4 mm and allowed to solidify and punched with a sterile cork borer (6.0 mm diameter) to make open cavities. Each plate should have maximum seven cavities with appropriate distances.

Preparation of test and standard solutions:

The stock solution of test was prepared by dissolving the dried chloroform extracts of leaves of *Abutilon indicum (Linn.)* at concentration of 50,100,200,300 and 500µg/ml in dimethyl sulphoxide (DMSO). The stock solution of reference standards (Cefexime) was prepared at a concentration of 20µg/ml in sterile water. Antimicrobial activity was screened by adding 0.05 ml stock solution to each cup by micropipette. The 20µg/ml Cefexime was used as positive control and 0.05 ml of DMSO was used as negative control. Antimicrobial activity was screened by adding 0.05 ml of both test and standard solution to each cavity of the plate using micropipette. Each microbial culture was inoculated into three petri plates. All the plates were kept for 1 to 4 hours at room temperature and then incubate them for about 24 hours at the incubator (33-34°C). After incubation, the bacterial inhibition zone diameters were measured and take the average of inhibition zone diameters of three plates of each organism was noted.

ANTHELMINTIC ACTIVITY:

Alcoholic extract of the stems, ethyl acetate and carbon tetra chloride fraction of aqueous extracts from the leaves plant of *Abutilon indicum* were investigated for their anthelmintic activity against *Pheretima posthuma*. Various concentrations (10, 20, 40 and 80 mg/ml) of each extracts were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Albenzadole was included as a standard reference and saline water as control. The anthelmintic assay was carried as per the method of [20] with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [21-22]. The experimental design of the investigation was carried out in seventeen groups with six worms in each group and carried out in the following regimes.

Group 1: Normal saline.
Group 2: Albendazole 10 mg/ml.
Group 3: Albendazole 20 mg/ml
Group 4: Albendazole 40 mg/ml
Group 5: Albendazole 80 mg/ml
Group 6: Alcoholic extract of the stems 10 mg/ml
Group 7: Alcoholic extract of the stems 20 mg/ml
Group 8: Alcoholic extract of the stems 40 mg/ml.
Group 9: Alcoholic extract of the stems 80 mg/ml.
Group 10:Ethyl acetate fraction of aqueous extract of leaves 10 mg/ml
Group 11:Ethyl acetate fraction of aqueous extract of leaves 20 mg/ml
Group 12:Ethyl acetate fraction of aqueous extract of leaves 40 mg/ml
Group 13:Ethyl acetate fraction of aqueous extract of leaves 80 mg/ml

Group 14:Carbon tetra chloride fraction of aqueous extract of leaves 10 mg/ml
Group 15:Carbon tetra chloride fraction of aqueous extract of leaves 20 mg/ml
Group 16:Carbon tetra chloride fraction of aqueous extract of leaves 40 mg/ml
Group 17:Carbon tetra chloride fraction of aqueous extract of leaves 80 mg/ml

Evaluation of anthelmintic activity by observations made for the time taken for paralysis and death of individual worms up to four hours of test period. The mean paralysis time and mean lethal time of each extract was recorded. Paralysis said to be occurred when worms did not revive even in normal saline. Death was concluded when worm lost their motility followed with fading away of their body color.

RESULTS AND DISCUSSION

Antimicrobial activity:

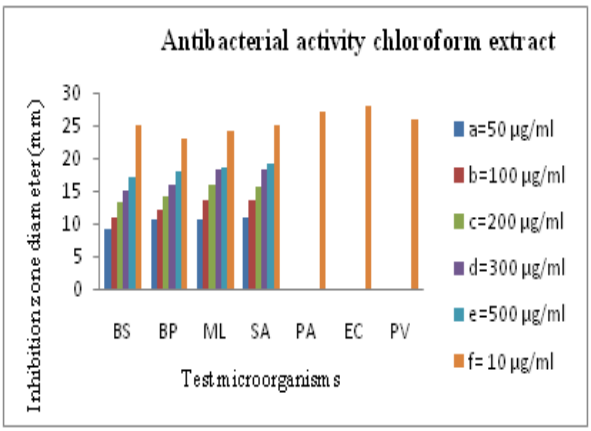
Chloroform extract of *Abutilon indicum* leaves showed antimicrobial activity against only on Gram +Ve microorganism. When compared within the microbial strains, chloroform extract showed inhibition zone diameter 19.3±0.5mm in diameter on *Staphylococcus aureus* NCIM 2079 at 500µg/ml. The extract showed growth inhibition zones against other strains in a dose dependent manner. But this extract did not show growth inhibition against Gram –Ve microorganisms, when compared with standard drug cefexime (10µg/ml). Here we are using a crude extract of plant. It is only preliminary activity for identification of antibacterial activities of the *Abutilon indicum.*

Table 1: Zone of inhibition of various concentrations of chloroform extract of *Abutilon indicum* leaves

Type of strain	Zone diameters (mm) with respect to Conc. of the Chloroform extract					Control	Conc. of Cefexime 10µg/ml
	50µg/m	100µg/ml	200µg/ml	300µg/ml	500µg/ml		
B.S	9±0.0	11±0.0	13.3±0.5	15±0.0	17±0.0	Nil	25
B.P	10.6±0.5	12±0.0	14±1.0	16±1.0	18.3±0.5	Nil	23
M.L	10.6±0.5	13.6±0.5	16±0.5	18.3±0.5	18.6±0.5	Nil	24
S.A	11±0.0	13.6±0.5	15.6±0.5	18.3±0.5	19.3±0.5	Nil	25
P.A	Nil	Nil	Nil	Nil	Nil	Nil	27
E.C	Nil	Nil	Nil	Nil	Nil	Nil	28
P.V	Nil	Nil	Nil	Nil	Nil	Nil	26

(Table 1) showed the inhibition zone diameters of various concentration of chloroform extract of the leaves versus standard concentration of Cefexime. The valves of each concentration of mean ± standard deviation (SD) of three replicates, Control: Dimethylsulphoxide (DMSO).B.S:*Bacillus subtilis* NCIM 2063*;* B.P:*Bacillus pumilis* NCIM 2327*;*M.L:*Micrococcus lutes NCIM 2871;* S.A*: Staphylococcus aureus* NCIM 2079*;*P.A*: Pseudomonas aeruginosa* NCIM 2037*;* E.C*: Escherichia coli* NCIM 2067*;*P.V*: Proteus vulgaris* NCIM 2027.

Figure 1: Antibacterial activity of Chloroform extract of leaves of *Abutilon indicum* at different concentrations against selected microbial strains



(Fig 1) shows antibacterial activity of Chloroform extract of leaves of *Abutilon indicum* at different concentrations against selected microbial strains. B.S:*Bacillus subtilis* NCIM 2063*;* B.P:*Bacillus pumilis* NCIM 2327*;*M.L:*Micrococcus lutes NCIM 2871;* S.A*: Staphylococcus aureus* NCIM 2079*;*P.A*: Pseudomonas aeruginosa* NCIM 2037*;* E.C*: Escherichia coli* NCIM 2067*;*P.V*: Proteus vulgaris* NCIM 2027

Antihelminthic activity:

The antihelminthic activity of alcoholic extract of *Abutilon indicum* stems showed good response on earth worms. The time taken for standard drug albendazole for paralysis of worms was 13±0.5 min and time taken for death iwas19±0.5min at 80mg/ml concentration. When compared with standard drug, alcoholic extract of stems showed time taken for paralysis was11±0.5min and time taken for death was 15±0.5min.at same dosage, like ethyl acetate fraction of aqueous extract of leaves, carbon tetra chloride fraction of aqueous extract of leaves also showed the time taken for paralysis 20±0.5, 38±0.5min and time taken for death was 27±0.5, 47±0.5 respectively at 80mg/ml concentration. antihelminthic activities of various extracts of leaves and stem are compared with Albenbdazole as standard. The valves of each

concentration of mean ± standard deviation (SD).*$P<0.05$, one way ANOVA followed by Dunnett's test (Paralysiis at 80 mg/kg).

Table 2: Antihelminthic activity of various extracts of leaves and stem compared with Albenbdazole as standard

Treatment	Concentration mg/ml of extract for each group	Time taken for paralysis (min)	Time taken for death (min)
Control (water)		Nil	Nil
Albendazole	10	27±0.5	37±0.5
	20	20±1.0	27±0.5
	40	17±0.5	22±0.5
	80	13±0.5	19±0.5
Alcoholic extract of the stems	10	25±1.0	30±1.0
	20	20±0.5	24±0.5
	40	15±0.0	21±0.5
	80	11±0.5^{ns}	15±0.5*
Ethyl acetate fraction of leaves	10	40±0.0	58±1.0
	20	35±1.0	37±0.5
	40	25±0.5	30±0.5
	80	20±0.5*	27±0.5*
Carbon tetra chloride fraction of leaves	10	65±0.5	70±1.0
	20	54±0.5	62±1.0
	40	47±1.0	59±0.5
	80	38±0.5*	47±0.5*

The valves of each concentration of mean ± standard deviation (SD).*$P<0.05$,one way ANOVA followed by Dunnett's test (Paralysiis at 80 mg/kg).

CONCLUSION

Chloroform extract of *Abutilon indicum leaves* showed antimicrobial activity against only on Gram +Ve microorganism. Like this, various extracts of this plant showed antihelminthic properties, when compared with standard albendazole. Further studies are needed for the confirmation of antibacterial action and antihelminthic activity of plant by isolating pure chemical constituents and also for the identification of the compound, that responsible for the properties of crude extract of the *Abutilon indicum.*

ACKNOWLEDGEMENT

The authors are thankful to the Management and Principal of NRI College of Pharmacy, Pothavarappadu, Agiripalli mandal, Krishna District, Andhra Pradesh, India, for the financial support (Ref no. NRIP/2012/Proj-22) and providing the research facilities.

REFERENCES

1. Kirtikar K R & Basu B D, Indian Medicinal Plants, Vol. I, (Reprint, Periodical Expert Book Agency, New Delhi), 1991; 314.
2. Bhakuni D S, Dhar M L, Dhar M M, Dhawan B N, Gupta B & Srimal R C, Screening of Indian plants for biological activity: Part III. Indian J Exp Biol, 1971; 9; 91.
3. Mokkhasmit M, Ngarnwathana W, Sawasdimongkol K & Permphiphat V, Pharmacological evaluation of Thai medicinal plants, J Med Assoc (Thialand),1971; 54;490.
4. Jain P K, Gharia A K, Sharma S K & Bokadia M M, Study of some essential oils for their antifungal activities, Indian Drugs, 1979; 16;122.
5. Dash G K, Samanta A, Kanungo S K, Sahu S K, Suresh P & Ganapaty S, Hepatoprotective activity of leaves of *Abutilon indicum*, Indian J Nat Prod, 2000; 16 (2); 25.
6. Chopra AK, Khanna DR, Prasad G, Malik DS, Bhutiani R. Medicinal Plants-Consevation, Cultivation and Utilization. Daya Publising House, Delhi, 2007; 19.
7. Khare CP. Indian medicinal plants, An Illustrated Dictionary. Springer-Verlag Heidelberg, New York, 2007; 3-4.
8. Kirtiar KR, Basu BD. Indian medicinal plants. Edition 3, Vol-I, sri satguru publication, Delhi India, 1983; 321-324.
9. Bagi MK, Kalyani GA, Dennis TJ, Kumar AK, Kakrani HK.A A preliminary pharmacological of *Abutilon indicum* Analgesic activity. Fitoterapia.1985; 56 (3): 169-171.
10. Seetharam G, Halageri S, Setty R, Bheemachar. Hypoglycemic activity of *Abutilon indicum* leaf extract in rats. Fitoterapia. 2000; 73 (2): 156-159.
11. Roshan S, Ali Sadath, Saleem MA, Patel MJ and Ali Asgar. Effect of *Abutilon indicum* on carbon tetrachloride induced hepatic damage in albino rats. Journal

Pharmacutical Research.2004; 3 (4): 61-62.
12. Ahamed M, Amin S, Islam M, Takahashi M, Okuyama E. Analgesic principal from *Abutilon indicum*. Die pharmazie.2000; 55 (4): 314-316.
13. Dhanalaksmi S, Lakshmanan KK, Subramanian MS. Chemical constituents from *Abutilon indicum*. J Res Edn Indian Med 1990; 9:21.
14. Sankara Subramanian S, Nair AGR. Flavonoids of four Malvaceous Plants. Phytochemistry 1972; 11:1518.
15. Sharma PV, Zafarul AA. TwoSesquiterpene Lactones from *Abutilon Indicum*. Phytochemistry 1989; 28:3525.
16. Irena, M. and Maria, S. Flavonoid compounds in the flowers of *Abutilon indicum* (L.) Sweet (Malvaceae). Acta Poloniae Pharmaceutica Drug Research, 2002; 59: 227-229.
17. Pengelly, A. Triterpinoids and saponins, in the constituent of medicinal plants, CABI publishing, USA, 2004; 74 PP.
18. Kashmiri, M.A., Yasmin, S., Ahmad, M. and Mohy-ud-Din, A.. Characterization, compositional studies, antioxidant and antibacterial activities of seeds of *Abutilon indicum* and Abutilon muticum grown wild in Pakistan, Acta Chimica Slovenica, 2009; 56: 345–352.
19. Trease and Evans, Pharmacognosy, Saunders Elsevier,16th Edition. 2009
20. Ajaiyeoba E.O., Onocha P.A. and Olarenwaju O.T. *In vitro* anthelmintic properties of *uchholzia coriaceae* and *Gynandropsis gynandra* extract. Pharm. Bio. 2001; 39:217-220.
21. Thorn G.W., Adams R.D., Braunwald E., Isselbacher K.J. and Petersdorf R.G. New York: Harrison's Principles of Internal Medicine. McGraw Hill Co. 1977.
22. Vidyarthi RD. A Text Book of Zoology. 14th edition. S. Chand and Co; New Delhi. 1967.