Asian Journal of Advances in Medical Science

1(1): 1-9, 2019

PHYTOCHEMICAL INVESTIGATION OF PLANTS *Acalypha indica* (L.) AND *Cocculus hirsutus* (L.) FROM PRAKASAM DISTRICT, ANDHRA PRADESH, INDIA

PASUMARTHI BRAHMAM[1] AND KANIKARAM SUNITA[1*]

[1]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar 522510, Guntur, Andhra Pradesh, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author PB managed the literature searches, analyzed the results, performed the statistical analyses and wrote the first draft of the manuscript. Author KS designed the study, wrote the protocol and managed the analyses of the study. Both authors read and approved the final manuscript.

Received: 02 August 2018
Accepted: 12 October 2018
Published: 19 October 2018

Original Research Article

ABSTRACT

Objectives: The present study report the phytochemical analysis of chloroform, ethyl acetate, methanolic extracts of leaf, stem bark and root of *Acalypha indica* (L.) and *Cocculus hirsutus* (L.) plants.
Methods: Plants material used were collected from Kadaparajupalle at Dornala Mandal, Prakasam district, Andhra Pradesh, India. The authentication of the plant species was done by the taxonomist. The plant part extraction was done by using soxhlet apparatus. The preliminary phytochemical screening of this extracts was conducted by following the standard methods for the presence of the alkaloids, saponins, terpenoids & steroids, flavonoids, tannins, phenolic compounds, coumarins, quinones, resins, and glycosides.
Results: Results indicated the presence of alkaloids, saponins, terpenoids & steroids, tannins, anthocyanidins, phenolic compounds, coumarins, quinones, resins and glycosides in all the plant extracts and could be used for the treatment of wounds and burns.
Conclusion: The presence of high alkaloids, flavonoids and terpenoids of the plant extracts suggest their antioxidant potential and justifies their therapeutic action which could be used for the drug formulation.

Keywords: Phytochemical constituents; *Acalypha indica*; *Cocculus hirsutus*; crude extracts.

1. INTRODUCTION

India is called Botanical Garden of the World and one of the world's twelfth leading biodiversity centres which contain over 45,000 different plant species, out of this, 15,000-20,000 are of good medicinal properties of which only about 7,000 - 7,500 are being used by traditional practitioners. There are about 25,000 licensed pharmacies of Indian system of medicine in India. At present, about 3000 compound formulations and 1000 single drugs are registered. Herbal industry in India uses about 8000 medicinal plants and an annual turnover of the Indian herbal medicinal industry is more lucrative. The Siddha medicine system uses around 600, Unani 700, Ayurveda 700, and modern medicine about

**Corresponding author: Email: drsunitamichael@gmail.com;*

30 plant species. After information technology, herbal technology is the India's biggest revenue source [1].

Generally plants play an important role in medicinal properties for both preventive and curative purposes. Phytochemicals are the plant derived substances, which have recently become great interest owing to their versatile application. In the traditional system of medicine, the medicinal plants are the richest bio-resource of drugs and it is also responsible for different flavours, colours, and smell. They also function as medicaments. These medicinal values of plants lie in some chemically active substance, that produce a definite physiological action the human body [2]. There are thousands of species of medicinal plants used globally for the cure of several infections. To find out its scientific basis, these plants are used as antimicrobial agents and several works have been carried out by scientists [3].

In the search of new drugs since ancient times, people have been exploring the nature particularly plants. This resulted in the use of large number of medicinal plants with curative properties to treat several diseases. For primary health care mostly 80% of the world relies on traditional medicine most of which involve the use of plant extracts. In India, 95% of the prescriptions were plant based in the traditional systems of Ayurveda, Siddha, Unani, and Homeopathy. The plant study continues principally for the discovery of novel secondary metabolites. Around 805 of the products were of plant origin and their sales exceeded US $65 billion in 2003 [4]. In regard to genetic resources of medicinal plants, India is the varietal emporium of medicinal plants and is one of the richest countries in the world. It exhibits wide range in topography and climate. Moreover, the agro- climatic conditions are conducive for introducing and domesticating new exotic plant varieties [5].

The plant *Acalypha indica,* which belongs to the family of Euphorbiaceae is a slender climbing of shrub that grows about six meters high in marshy places [6]. Nearly in the backyards of houses and waste places throughout the planes of India. Extracts of *A. indica* are used as emetic, laxative, diuretic expectorant, and for the treatment of bronchitis, pneumonia, asthma and pulmonary tuberculosis. The plant is used in homeopathy to treat severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis (PTB) [7].

Cocculus hirsutus belongs to the family Menispermaceae, a perennial climber that can form a dense cover on top of other plants mainly found in tropical and subtropical climatic conditions [8]. In India it is found almost throughout in open habitats and dry localities including Uttar Pradesh, Karnataka, Gujarat, Orissa, Tamilnadu, Rajasthan, Bihar, Maharashtra and West Bengal. It has a special potency as a detoxifier. The leaves are useful in gonorrhoea, cough, ophthalmia, cephalalgia, and neuralgia and also used to treat skin infections and itchy skin including rheumatism [9]. For the treatment night blindness, the cooked leaves are used in Rajasthan (India). The juice of leaves is used externally as a cooling and smoothing agent in eczema, impetigo. Different aerial parts of the plant report to be used as a diuretic, laxative [10] and root extract showed analgesic and anti- inflammatory effect [11].

The phytochemical investigation plays a vital role in identifying new sources of therapeutically and industrially important compounds like alkaloids, phenolic compounds, steroids, flavonoids, tannins, saponins, terpenoids etc. [12]. So the present study was aimed to determine the phytochemical compounds present in the chloroform, ethyl acetate and methanolic extracts of different parts of plants *A. indica* and *C. hirsutus.*

2. MATERIALS AND METHODS

Plants parts (leaf, stem bark and root) of *A. indica* and *C. hirsutus* were collected from a place called Kadaparajupalle at Dornala Mandal, Prakasam District, Andhra Pradesh, India. Prakasam District is the one of the southernmost districts of Andhra Pradesh lies between 14˚ 57' and 16˚ 17' North latitude and 73˚ 43' and 80˚ 25' East longitude, occupying an area about 17,626 Sq. Km. The total population of the district is 33.84,192. The Nallamala is and the Veligondla are the two major hill ranges in the district, of which Vermakonda situated in the Eastern Nallamala has the highest peak (939 m). The Nallamala is hills which form a part of Eastern Ghats run through this district is distributed by several medicinal plants which are used traditionally by local tribal people [13].

The authentication of the plant species was done by taxonomist Prof. Dr Vatsavaya S. Raju, Department of Botany Kakatiya University, Warangal, Telangana state, India. The plants *A. indica* and *C. hirsutus* were deposited in the Department of Botany, Kakatiya University and Voucher numbers were given as *Acalypha indica* L. – Acc. no. KUW1927 of Euphorbiaceae and *Cocculus hirsutus* (L.) W. Theob. – Acc. no. KUW1925 of Menispermaceae.

All the collected plant parts were washed thrice with tap water twice with distilled water to remove the dirt and adhering materials.

2.1 Preparation of Plant Extracts

Selected parts of two plants were collected and left at room temperature for two weeks to dry. Samples were chopped into smaller pieces and then ground into powder. The samples were then stored in jars at room temperature until extraction. Shade- dried medicinal plant samples were subjected to extraction in 90% of different organic solvents i.e., chloroform (60-62°C), ethyl acetate (76-77°C), and methanol (65°C) in a soxhlet apparatus (Borosil). For *A. indica* plant part extraction; 100 g of leaf 250 g of stem bark and 350 g of root in the form of powdered material was weighted accurately and used for the extraction in the above solvents. For *C. hirsutus* plant part extraction; 150 g of leaf, 170 g of stem bark and 250 g of root in the form powdered material was used for the extraction of chloroform, ethyl acetate and methanol. After complete extraction, the filtrates were concentrated separately by rotary vacuum evaporation (>45°C) and then freeze dried (-20°C) to obtain a solid residue. The extraction percentage was calculated by using the following formula:

$$\text{Percentage of extraction} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material (g)}} \times 100$$

These plant extracts were then screened for the presence of phytochemical constituents by the standard methods.

2.2 Phytochemical Analysis

Chemical test were carried out using various extracts such as chloroform, ethyl acetate and methanol. The phytochemical analysis of plant extract for the presence of alkaloids, saponins, terpenoids, steroids, tannins, anthocynidins, phenolic compounds, flavonoids, coumarins, quinines, resins and glycosides was done by following the standard methods according to Sofowara and Harbone [14,15].

2.2.1 Test for alkaloids

The plant extract was evaporated to dryness and the residue is dissolved in 1% HCl. To the solution, Mayer's and Dragandroff's reagents were added. Appearance of precipitate or turbidity indicated the presence of alkaloids.

a. **Mayer's reagent:** 1.3 g of HgCl and 5 g of KI were dissolved separately in 60 ml of double distilled water respectively and both solutions were mixed and diluted to 100 ml.
b. **Dragandroff's reagent:** 8 g of Bismuth nitrate was dissolved in 20 ml of Concentrated HNO_3 and 27.2 g of KI in 50 ml of double distilled water. Both the solutions were allowed to stand still KIO_3 crystallised out. Supernatant was decanted and the final volume was adjusted to 100 ml.

2.2.2 Test for saponins

The plant extract was evaporated to dryness**.** Tap water was added and shaken vigorously. Formation of persistent foam of about 2 cm was taken as a positive reaction.

2.2.3 Test for terpenoids and steroids

A volume of 50% of H_2SO_4 was added along the sides of test tube containing a mixture of methanolic HCl and anhydride. The change in colour form green to blue green (sometimes via red or blue) indicated the terpenoids and steroids.

2.2.4 Test for tannins

The plant extract was evaporated to dryness and the residue was dissolved in water and tested with 1% gelatine solution and gelatine salt solution. (1 g gelatine dissolved in 10 g of NaCl (w/w) to separates volumes). The appearance of white precipitate was regarded as positive reaction.

2.2.5 Test for anthocyanidins

The plant extract was added with equal volume of HCl. Appearance of red or purple colour indicates the presence of anthocynidins.

2.2.6 Test for phenolic compounds

The formation of intense colour in the plant extract by adding 1-2 drops of 1% ferric chloride to the extract was considered as a positive reaction for phenolic compounds.

2.2.7 Test for the flavonoids

To the plant extract, Conc. HCl and Mg powder were added. The presence of flavonoids was identified by the development of pink or magenta or red coloured foam.

2.2.8 Test for coumarins

To the plant extract, a few drops of alcoholic sodium hydroxide were added. Formation of yellow colour that indicated the presence of coumarins.

2.2.9 Test for quinones

To the 1 ml of plant extract, 1 ml of Conc. H_2SO_4 was added. Formation of the red coloured showed the presence of Quinones.

2.2.10 Test for resins

Plant extract were treated with acetone. To this, small amount of water was added and shaken. The appearance of turbidity indicated the presence of resins.

2.2.11 Test for glycosides

a. The plant extract was mixed with a little anthrone on a watch glass. Few drops of Conc. H_2SO_4 was added and warmed gently over water bath. The presence of glycosides was identified by dark green colour formation.

b. To the plant extract few drops of glacial acetic acid, ferric chloride and Conc. H_2SO_4 were added and was observed for the formation of reddish brown coloration at the junction of the two layers and the bluish green colour in upper layer [16].

3. RESULTS

The different plant part extracts that were prepared from plants *A. indica* and *C. hirsutus* (Fig. 1 and Fig. 2) were subjected to phytochemical analysis for the presence of different bioactive compounds.

The amount and percentage yield of chloroform leaf extract was 3 g (3%), ethyl acetate leaf extract was 4 g (4%) and methanol leaf extract was 5 g (5%) which were extracted from 100 g of leaf powder of *A. indica*. The amount and percentage yield of chloroform stem bark extract was 2 g (0.8%), ethyl acetate stem bark extract was 2 g (0.8%) and methanol leaf extract was 4 g (1.6%) which were extracted from 250 g of stem bark powder of *A. indica*. The amount and percentage yield of chloroform root extract was 1.5 g (0.43%), ethyl acetate leaf extract was 1.7 g (0.48%) and methanol leaf extract was 2 g (0.57%) which were extracted from 350 g of root powder each. Among all these, more percentage yield was obtained with methanolic leaf extract (5.0%) of *A. indica* (Table 1). Phytochemical screening of the chloroform, ethyl acetate and methanolic extracts of leaves, stem bark and roots of *A. indica* have shown the presence of various medicinally active constituents and represented in Table 2. A total of 11 phytochemicals were analysed. The chloroform leaf extract of *A. indica* showed the presence of 6 phytochemicals, ethyl acetate leaf extract showed 2 compounds and methanol leaf extract showed the presence of 4 compounds. The chloroform stem bark extract showed the presence of 6 phytochemicals, ethyl acetate stem bark extract showed the presence of only 1 compound.and methanolic stem bark extract showed the presence of 2 compounds. The chloroform root extract showed the presence of 3 phytochemicals, ethyl acetate root extracts showed the presence of 3 compounds and also methanolic root extract showed the presence of 3 compounds. Phenolic compounds and glycosides were present in all the solvent extracts of the leaf. Coumarins and quinones were present in all three solvent extracts of root and phenolic compounds were also present in all three solvent extracts of leaf (Table 2).

The amount and percentage yield of chloroform leaf extract was 2.5 g (1.7%), ethyl acetate leaf extract was 2.9 g (1.9%) and methanol leaf extract was 11 g (7.3%) which were extracted from 150 g of leaf powder of C. *hirsutus*. The amount and percentage yield of chloroform stem bark extract was 1.3 g (0.8%), ethyl acetate stem bark extract was 1.7 g (1.0%) and methanol leaf extract was 11 g (6.5%) which were extracted from 170 g of stem bark powder of C. *hirsutus*. The amount and percentage yield of chloroform root extract was 1.5 g (0.7%), ethyl acetate leaf extract was 2 g (0.8%) and methanol leaf extract was 10 g (4.0%) which were extracted from 350 g of root powder each. Among all these, more percentage yield was obtained with methanolic leaf extract (7.3%) of C. *hirsutus* (Table 3).

Phytochemical screening of the chloroform, ethyl acetate and methanolic extracts of *C. hirsutus* leaves, stem bark and roots have shown the presence of various medicinally active constituents and represented in Table 4. A total of 11 phytochemicals were analysed. The chloroform leaf extract of *C. hirsutus* showed the presence of 2 compounds, ethyl acetate leaf extract showed the presence of 3 compounds, methanol leaf extract showed the presence of 4 phytochemicals. The chloroform stem bark extract showed the presence of 2 compounds, ethyl acetate extract of stem bark showed the presence of 2 compounds and methanol extract of stem bark showed the presence of 3 phytochemical compounds. The chloroform root extract showed the presence of 3 compounds, ethyl acetate extract of root showed the presence of 2 compounds and methanol extract of root showed the presence of 4 phytochemical compounds. Phenolic compounds were present in all solvent

extracts of the leaves and stem bark. Alkaloids were present in all solvent extracts of the stem bark and root. Phenolic compounds were present in three solvent extracts of leaves and stem bark. Quinones were present in three solvent extracts of root and Glycosides were present in three solvent extracts of leaves of *C. hirsutus* (Table 4).

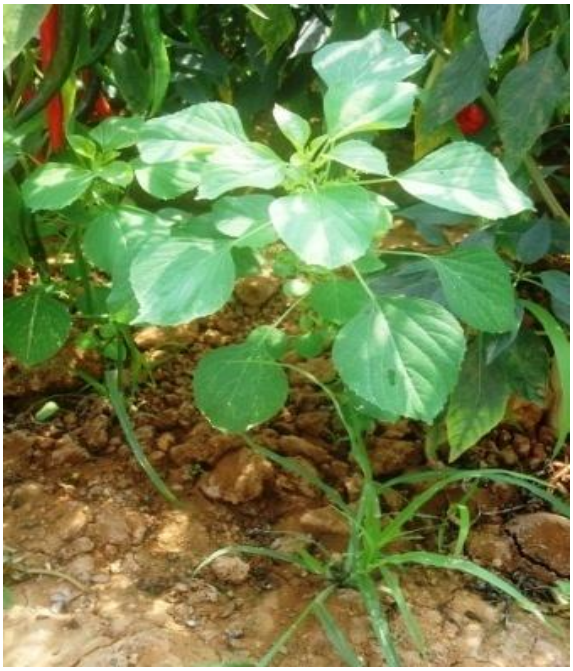

Fig. 1. *Acalypha indica* (L.) whole plant

Fig. 2. *Cocculus hirsutus* (L.) whole plant

4. DISCUSSION

Several studies have proved that the phytochemicals present in a medicinal plant are widely responsible for the therapeutic potential of the plant. According to the World Health Organisation, medicinal plants would be the best source to obtain a variety of drugs [17].

The results of phytochemical screening showed that the chloroform extracts of *A. indica* contain alkaloids, saponins, terpenoids & steroids, tannins, phenolic compounds, coumarins, quinines, resins and glycosides. Ethyl acetate extracts of *Acalypha indica* contain phenolic compounds, coumarins, quinines, and glycosides. Methanol extracts of *A. indica* contain alkaloids, tannins, phenolic compounds, coumarins, quinines, and glycosides.

Similar work was done by Pragada et al. [18] who did phytochemical screening and reported that the Hydro-alcoholic crude extract of *A. indica* showed a positive test for flavonoids, steroids, tannins, amino acids and oils. The methanolic fraction of *A. indica* showed a positive test for flavonoids, saponins, amino acids and oils. The ethyl acetate fraction of *A. indica* showed positive test for tannins, steroids, amino acids and oils. The hexane fraction of *A. indica* showed positive test for steroids, oils and amino acids.

The present work is supported by Kumarasamyraja et al. [19] who identified the phytochemical constituents in petroleum ether, chloroform, ethyl acetate and methanolic leaf extracts of extracts of *A. indica.* Petroleum ether extract of leaf had shown fixed oil, gum and mucilage. Chloroform extract of leaf showed alkaloids, phytosterols, tannins, phenols, flavonoids, gum & mucilage and saponins. Ethyl acetate extract of leaf showed only flavonoids. Methanol extract of leaf has shown tannins, phenols and saponins which is in correlation with our study.

Paindla and Mamidala [20] have conducted phyotochemical screening of *A. indica* leaf extractions in different solvents such as hexane, chloroform, ethyl acetate, acetone and methanol which showed the presence of carbohydrates, alkaloids, starch, proteins and glycosides; slightly the presence of phenols and tannins, but it gave the negative for results to saponins. Among these phytochemicals, alkaloids, carbohydrates, starch, glycosides were present in all extracts. Phenols were present in hexane, chloroform and acetone extracts. Tannins were present in hexane, chloroform and acetone extracts. The above study supports the present findings that the leaf extract contains alkaloids, saponins, terpenoids & steroids, tannins, phenolic compounds and glycosides.

Table 1. Percentage yield of crude extracts of leaf, stem bark and root of *Acalypha indica*

Plant part	Solvent	Initial weight (g)	Yield of the extract (g)	Percentage of yield (%)
Leaf	Chloroform	100	3	3.0
	Ethyl Acetate	100	4	4.0
	Methanol	100	5	5.0
Stem bark	Chloroform	250	2	0.8
	Ethyl Acetate	250	2	0.8
	Methanol	250	4	1.6
Root	Chloroform	350	1.5	0.43
	Ethyl Acetate	350	1.7	0.48
	Methanol	350	2	0.57

Table 2. Phytochemical investigation of crude extracts of leaf, stem bark and root of *Acalypha indica*

Phytochemical constituent	Leaf			Stem bark			Root		
	C	EA	M	C	EA	M	C	EA	M
Alkaloids	+	-	-	+	-	+	-	-	+
Saponins	+	-	-	+	-	-	+	-	-
Terpenoids and Steroids	+	-	-	+	-	-	-	-	-
Tannins	+	-	+	+	-	-	-	-	-
Anthocyanidins	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	+	+	+	+	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	+	+	+
Quinones	-	-	-	-	-	-	+	+	+
Resins	-	-	+	-	-	-	-	-	-
Glycosides	+	+	+	+	-	+	-	+	-

+ = Presence, - = Absence, C = Chloroform, EA = Ethyl acetate and M = Methanol

Table 3. Percentage yield of crude extracts of leaf, stem bark and root of *Cocculus hirsutus*

Plant part	Solvent	Initial weight (g)	Yield of the extract (g)	Percentage of yield (%)
Leaf	Chloroform	150	2.5	1.7
	Ethyl acetate	150	2.9	1.9
	Methanol	150	11	7.3
Stem bark	Chloroform	170	1.3	0.8
	Ethyl acetate	170	1.7	1.0
	Methanol	170	11	6.5
Root	Chloroform	250	1.5	0.7
	Ethyl acetate	250	2	0.8
	Methanol	250	10	4.0

Table 4. Phytochemical investigation of crude extracts of leaf, stem bark and root of *Cocculus hirsutus*

Phytochemical	Leaf			Stem bark			Root		
	C	EA	M	C	EA	M	C	EA	M
Alkaloids	-	+	+	-	+	+	+	+	+
Saponins	-	-	-	+	-	-	-	-	-
Terpenoids and Steroids	-	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-	-	-
Anthocyanidins	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	+	+	+	+	+	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	-	-	+
Quinones	-	-	-	-	-	-	+	+	+
Resins	-	-	+	-	-	-	+	-	+
Glycosides	+	+	+	-	-	+	-	-	-

+ = Presence, - = Absence, C = Chloroform, EA = Ethyl acetate and M = Methanol

Similar work was done by Kumar et al. [21] that the powdered whole plant of *A. indica* was individually extracted with different solvents such as hexane, chloroform, ethyl acetate and methanol. The whole plant extract of the *A. indica*, showed the presence of glycosides, alkaloids, tannins, phenols, steroids and saponins but in our study we observed alkaloids, saponins, terpenoids & steroids, tannins, phenolic compounds, flavonoids, resins and glycosides in aerial parts (leaf, stem bark and root) of *A. indica*.

The results of phytochemical screening showed that the chloroform extracts of *C. hirsutus* contains alkaloids, saponins, phenolic compounds, quinines, resins and glycosides. Ethyl acetate extracts of *C. hirsutus* contain alkaloids, phenolic compounds, quinines, and glycosides. Methanol extracts of *C. hirsutus* contain alkaloids, phenolic compounds, coumarins, quinines, resins and glycosides. Similar work was done by Patil et al. [22] and World Health Organization (WHO) reported the phytochemical analysis of different solvents of *C. hirsutus* plant. Petroleum ether showed positive test for oils and fats. Chloroform extract showed positive tests for alkaloids, glycosides, steroids, saponins, oil & fats and phenolic compound and tannins. Alcohol extract showed positive tests for saponins, steroids, oils & fats, phenolic compounds and tannins which is in correlation with our study.

Similarly, peer researcher Meena et al. [23] did phytochemical investigation of methanolic extract of *C. hirsutus* leaves which showed the presence of alkaloids, carbohydrates, glycosides, steroids, flavonoids, saponins and tannins. Whereas in the present study, the presence of alkaloids, phenolic compounds, resins and glycosides in leaf extraction of *C. hirsutus* was observed. Thus, our study is in correlation with the peer researchers and our results confirm the earlier work. Hence, the plants *A. indica* and *C. hirsutus* have shown various secondary metabolites which indicate the therapeutic potential for different ailments.

5. CONCLUSION

Mostly plants play a major role in the traditional medicinal system to combat several diseases. Generally, plants have many phytochemicals like alkaloids, saponins, terpenoids and steroids, tannins, anthocyanidins, phenolic compounds, flavonoids, coumarins, quinones resins and glycosides with specialised properties. The plants screened for phytochemical analysis seemed to have the potential to act as a resource of drugs. Thus the study justifies the use of these plant species in medicinal field.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

I am thankful to the Co-ordinator, Deportment of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh for providing necessary facilities through UGC-SAP-DRS – Phase III and UGC-FIST – Phase I funding. The main author is highly thankful to UGC, New Delhi for providing UGC-BSR fellowship to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sharma A, Shanker C, Tyagi L, Singh M, Rao CV. Herbal medicine for market potential in India: An overview. Acad. J. Plant Sci. 2008;1(2):26-36.
2. Karunyadevi S, Arun N, Surekha V. Screening of phytochemical compounds, antioxidant and antimicrobial activity of *Aloe vera* and Arkaa. Adv. Biotech. 2009;9:38-43.
3. Omotayo AE. Antibacterial activity of some antimalarial plants. Proc. Niger. Soc. Microbiol. 1998;39:69-72.
4. Patwardhan B, Ashok DBV, Mukund C. Ayurveda and natural products discovery. Curr. Sci. 2004;86(6):789-799.
5. Martins AP, Salgueiro L, Goncalves MJ, da Cunha AP, Vila R, Cañigueral S, Mazzoni V, Tomi F, Casanova J. Essential oil composition and antimicrobial activity of three Zingiberaceae from S. Tomé e Principe. Planta Med. 2001;67(6):580-584.
6. Noumedem JAK, Tamokou J. de D, Teke GN, Momo RCD, Kuete V, Kuiate JR. Phytochemical analysis, antimicrobial and radical-scavenging properties of *Acalypha manniana* leaves. SpringerPlus. 2013;2:503.
7. Yaakob Z, Ali E, Zainal A, Mohamad M, Takriff MS. An overview: Biomolecules from microalgae for animal feed and aquaculture. J. Biol. Res. (Thessalon). 2014;21(1):6.
8. Panda BR, Mohanta SR, Mishra US, Kar S, Panda BK, Chakraborty P. Antibacterial activity of the leaves of *Cocculus hirsutus*. Indian Drugs. 2007;44(2):108-110.
9. Chadha YR. The wealth of India: A dictionary of Indian raw materials and industrial products. Ind. Med. Gaz., NISCIR, CSIR, New Delhi. 1950;258.
10. Ganapaty S, Vijay K. Hypoglycemic activity of aerial parts of *Cocculus hirsutus alloxan* induced diabetes. Ind. J. Nat. Prod. 2006;22(4): 17-20.
11. Nayak SK, Singhai AK. Anti-inflammatory and analgesic activity of roots of *C. hirsutus*. Ind. J. Nat. Prod. 1993;9:12-14.
12. Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extract of *Byrosocarpus coccineus*. Fitoterapia. 2007; 78(1):25-28.
13. Mohan RK, Murthy PB. Plants used in traditional medicine by Tribals of Prakasam district, Andhra Pradesh. Anc. Sci. Life. 1992;11(3-4):176.
14. Sofowara A. Medicinal plants and traditional medicine in Africa, Spectrum Books Ltd., Nigeria. 1993;289.
15. Harborne JB. Phytochemical methods. Chapmed & Hall Ltd., London. 1973;49-188.
16. Siddiqui AA, Ali M. Practical pharmaceutical chemistry. 1st Ed.; CBS Publishers and Distributors, New Delhi. 1997;126-131.
17. Arunkumar S, Muthuselvam. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. Against clinical pathogens. World J Agril Sci. 2009;5(5):572-576.
18. Pragada R, Vangepurapu V, Ethadi SR, Praneeth VS. Phytochemical investigation and in vitro antioxidant, antimicrobial activity of different fractions of *Acalypha indica* Linn. Int. J. Pharm. Sci. 2011;3(4):314-317.
19. Kumarasamyraja D, Jeganathan NS, Manavalan R. Phytochemical investigation and antimicrobial activity of *Acalypha indica* Linn. Novel Science Int. J. Pharm. Sci. 2012;1(6): 313-316.

20. Paindla P, Mamidala E. Phytochemical and chromatographic studies in the leaves extract of *Acalypha indica*. Online International Interdisciplinary Res. J. 2014;4(1).
21. Kumar PK, Nirmala Babu Rao, Rani AR. Antimicrobial, antioxidant activity and phytochemical screening of *Acalypha indica* crude leaf extract. Int. J. Pharm. Clin. Res. 2016;8(6):583-588
22. Patil SA, Sujaya M, Patil SB. Aphrodisiac and phytochemical studies of *Cocculus hirsutus* extracts in albino rats. Asian Pac. J. Reprod. 2014;3(1):23-29.
23. Meena MK, Singh N, Patni V. Determination of bioactive components of the leaves of *Cocculus hirsutus* (L.) diels using GC-MS analysis. Int. J. Pharm. Pharm. Sci. 2014;6: 327-329.