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Phytochemical Investigation and In vitro Antimalarial Activity of *Acalypha indica* (L.) and *Cocculus hirsutus* (L.) From Prakasam District, Andhra Pradesh, India

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The present study, report the phytochemical analysis and *invitro* antimalarial activity of plants *Acalypha indica* (L.) and *Cocculus hirsutus* (L.). The *A. indica and C. hirsutus* plant was collected from Kadaparajupalle at Dornalamandal, Prakasam district, Andhra Pradesh, India. Leaf, stem bark and root crude extracts prepared in Soxhlet apparatus with chloroform, ethyl acetate and methanol solvents. The preliminary phytochemical screening of these extracts was conducted by following the standard methods. These extracts were tested for *in vitro*anti malarial activity against 3D7 and K1 strains of *Plasmodium falciparum* by standard laboratory protocol. *In vitro*cyto toxicity of the extracts was also tested by following standard laboratory method. The phytochemical screening has revealed the presence of alkaloids, saponins, terpenoids & steroids, tannins, anthocyanidins, phenolic compounds, coumarins, quinones, resins and glycosides. Amongst all the extracts screened for antimalarial activity, the leaf chloroform and ethyl acetate extracts of *A. indica* shown IC_{50} values of 3.34 μg/mL and 3.71 μg/mL respectively against 3D7 strain; the leaf chloroform and ethyl acetate extracts of *A. indica* shown IC_{50} values of 1.47 μg/mL and 2.32 μg/mL respectively against K1 strain; the root chloroform and methanol extracts of *C. hirsutus* shown IC_{50} values of $<$0.78 μg/mL and 3.714 μg/mL respectively against 3D7 strain; the root chloroform and methanol extracts of *C. hirsutus* shown IC_{50} values of $<$0.78 μg/mL and 2.10 μg/mL respectively against K1 strain. Thus, the above extracts have shown very active antimalarial activity against 3D7 and K1 strains. And all the extracts were non-toxic showing CC_{50} values of $>$20 μg/mL against Vero cell line. 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. The chloroform root extract of *C. hirsutus* has shown excellent antimalarial activity which can be used for the development of new antimalarial drug policies.

Keywords: *Acalypha indica*,*Cocculus hirsutus*, Crude extracts, Antimalarialactivity, Cytotoxicity.

India is called Botanical Garden of the World and one of the world's twelfth leading biodiversity centres which contains over 45,000 different plant species, out of this, 15,000-20,000 species are 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

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India uses about 8000 medicinal plants and 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 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 have recently become great interest owing to their versatile application. In traditional system of medicinethe medicinal plants are richest bio-resource of drugs and it also responsible for different flavours,colours, and smell. They also functions as medicaments. These medicinal values of plants laid in some chemically active substance that produces a definite physiological action in the human body.[2] There are 1000 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. All most all in India, 95% 0f 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 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 conductive for introducing and domesticating new exotic plant varieties.[5]

The plant *Acalyphaindica,* which belongs to the family of Euphorbiaceaeis 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 habits 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 usedin 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 antiinflammatory effect.[11]

In the early 2000s *Plasmodium falciparum* parasites resistant to mefloquine, chloroquine (CQ), quinine, proguanil, atovaquone and sulphadoxine-pyramethamine, but not artemisinins were reported.[13] The introduction of artemisinin-based combination therapy has resulted in a substantial decline in malaria during the last decade. However, the recent gains in antimalarial therapy are threatened by the emerging artemisinin resistant *P. falciparum* malaria in Southeast Asia. Consequently, new drugs and drug combinations are urgently needed for the treatment of malaria. Ideally these drugs should have novel modes of action and be chemically different from drugs in current use.[14]

The plants *A. indica* and *C. hirsutus* were selected for the study, because the first one grows along the road side and second one grows in forests of India and other tropical and subtropical regions. In Andhra Pradesh, these are commonly available and used as ethnomedicinal plants in Nallamalais forest in Prakasam District, Andhra Pradesh, India. [15]

Hence, in the present study, the leaf, stem bark and root extracts of *A. indica and C. hirsutus* were screened for phytochemical constituents.

The phytochemical investigation plays vital role in identifying new sources of therapeutically and industrially important compounds like alkaloids, phenolic compounds, steroids, flavonoids, tannins, saponins, terpenoidsetc.[12] So the present investigation aimed to identify the phytochemical compounds and to evaluate the antimalarial activity of the crude extracts of *A.indica* and *C. hirsutus.* These extracts were tested for *in vitro* antimalarial activity against CQ - sensitive 3D7 and CQ - resistant K1 strains of *P. falciparum*. And all these extracts were screened for cytotoxicity against Vero cell line.

MATERIALS AND METHODS

Collection of Plant Material

Plants were collected from a place called Kadaparajupalle at Dornala mandal, Prakasam district, Andhra Pradesh, India. Prakasam district is 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 Nallamalais and the Veligondla are the two major hill ranges in the district, of which Vermakonda situated in the Eastern Nallamalais has the highest peak (939 m). The Nallamalais 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.[16]

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 *Cocculushirsutus* (L.) W. Theob. – Acc. no. KUW1925 of Menispermaceae.

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

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 for in 90% 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 solid residue. The extraction percentage was calculated by using the following formula:

Percentage of extraction = (Weight of the extract (g))/(Weight of the plant material (g))× 100

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

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.[17,18]

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.

Mayer's reagent

1.3 g of $HgCl_2$ 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.

Dragandroff's reagent

8 g of Bismuth Nitrate was dissolved in 20 ml of Conc. HNO_3 and 27.2 g of KI in 50 ml of double distilled water. Both the solutions were allowed to stand still and then $KIO_3$was crystallized out. Supernatant was decanted and the final volume was adjusted to 100 ml.

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

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

Table 1. Percentage yield of different crude extracts of *Acalyphaindica*

Plant part	Solvent	Initial Weight (g)	Yield of the extract (g)	Percentage of yield (%)
Leaf	Chloroform	100	3	3
	Ethyl Acetate	100	4	4
	Methanol	100	5	5
Stembark	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 investigationof different extracts of leaf, stem bark and root of *Acalypha indica*

Phytochemical	Leaf			Stem bark			Root		
Compound	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

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 opposite reaction.

Test for Terpenoids and Steroids

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

Test for Tannins

The plant extract was evaporated to dryness and the residue was dissolved in water and tested with 1% 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.

Test for Anthocyanidins

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

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.

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.

Test for Coumarins

To the plant extract, a few drops of alcoholic Sodium Hydroxide were added.

Table 3. Percentage yield of different crude extracts of *Cocculus hirsutus*

Plant part	Solvent	Initial Weight (g)	Yield of the extract (g)	Percentage of yield (%)
Leaves	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
	Methanol	170	11	6.5
Root	Chloroform	250	1.5	0.7
	Ethyl Acetate	250	2	0.8
	Methanol	250	10	4

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

Phytochemical	Leaves			Stem bark			Roots		
Compound	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

Formation of yellow colour indicated the presence of coumarins.

Test for Quinones

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

Test for Resins

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

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 observed for the formation of reddish brown coloration at the junction of the two layers and the bluish green colour in the upper layer.[19]

In vitro* cultivation of *Plasmodium falciparum

The *in vitro* cultures of both CQ -sensitive (3D7) and CQ -resistant (K1) strains of *P. falciparum* are routinely maintained in medium

Table 5. Antimalarial activity against CQ-sensitive *P. falciparum* 3D7strain & CQ-resistant *P. falciparum* K1 strain and cytotoxicity against Vero cell line of different plant part extracts of *Acalypha indica*

Plant part	Extract solvent	IC_{50} against *Plasmodium falciparum* 3D7 Chloroquine sensitive strain (µg/mL)	IC_{50} against *Plasmodium falciparum* K1 Chloroquine resistant strain (µg/mL)	CC_{50} against Vero cell line (µg/mL)
Leaf	chloroform	3.34	1.47	>500
	Ethyl acetate	3.71	2.32	490.34
	methanol	32.85	23.91	>500
Stem bark	chloroform	14.75	12.25	>500
	Ethyl acetate	19.07	10.63	>500
	methanol	14.07	7.92	>500
Root	chloroform	19.44	12.13	373.6
	Ethyl acetate	23.54	9.64	>500
	methanol	39.21	15.69	>500

Table 6. Antimalarial activity against CQ-sensitive *P. falciparum* 3D7strain & CQ- resistant *P. falciparum* K1 strain and cytotoxicity against Vero cell line of different plant part extracts of *Cocculus hirsutus*

Plant part	Extract solvent	IC_{50} against *Plasmodium falciparum* 3D7 Chloroquine sensitive strain (µg/mL)	IC_{50} against *Plasmodium falciparum* K1 Chloroquine resistant strain (µg/mL)	CC_{50} against Vero cell line (µg/mL)
Leaf	chloroform	16.74	5.08	>500
	Ethyl acetate	15.47	6.81	474.36
	methanol	>50	38.19	>500
Stem bark	chloroform	9.28	6.33	>500
	Ethyl acetate	8.10	4.54	>500
	methanol	15.41	12.32	>500
Root	chloroform	<0.78	<0.78	355.94
	Ethyl acetate	7.33	4.72	>500
	methanol	3.34	2.10	410.09

RPMI supplemented with 25 mM HEPES, 0.2% D-glucose, 0.21% Sodium Bicarbonate and 0.5% ALBUMAX-II.[20]The stock (5 mg/mL) solution of compound was prepared in DMSO and required dilutions were prepared in culture medium. For evaluation of 50% Inhibitory Concentration (IC_{50}) of the compound, Malaria SYBR Green-1 based fluorescence (MSF) assay[21] was carried out.

***In vitro* Antimalarial assay**

The highest concentration of extract used was 50.0 μg/mL. Two-fold serial dilutions of test sample were made in 96 well plate and incubated with 1.0% parasitized cell suspension containing 0.8% parasitaemia (Asynchronous culture with more than 80% ring stages). The plates were incubated at 37°C in CO_2 incubator in an atmosphere of 5% CO_2 and air mixture. 72 hours later 100 μL of lysis buffer containing 2x concentration of SYBR Green-1(Invitrogen) was added to each well and incubated for one hour at 37°C. The plate was examined at 485±20 nm of excitation and 530±20 nm of emission for relative fluorescence units (RFUs) per well using the Fluorescence Plate Reader (FLX800, BIOTEK). The IC_{50} values were obtained by Logit Regression Analysis of dose-response curves. Chloroquinediphosphate (SIGMA) was used as the reference drug.

The *in vitro* antimalarial activity was analysed in accordance with the norms of antimalarial activity of Rasoanaivo *et al.*[22] According to this norm, an extract is very activeif IC_{50}<5 μg/mL, active if 5 to <50 μg/mL, weakly active if 50 to <100 μg/mL, inactive if >100 μg/mL.

Criteria for selection of promising lead: IC_{50} = 10.0 μg/mL

Evaluation of *In vitro* Cytotoxicity assay

Cytotoxicity of test samples was carried out using Vero cell line (C1008; Monkey kidney fibroblast cells) following the method as mentioned is Sharma *et al.*[23] The cells were incubated with test sample dilutions for 72 h and MTT was used as reagent for detection of cytotoxic activity. The highest concentration of test samples used was 100μg/mL. 50% cytotoxic concentration (CC_{50}) was determined using dose–response curves. Podophyllotoxin (SIGMA) was used as the reference drug.An extract was classified as non-toxic when the CC_{50} value is >20 μg/mL.

Selectivity Index (SI) can be calculated as: SI = CC_{50} / IC_{50}

Criteria for selection: SI = >50.0

RESULTS

The plant extracts which 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 of extract obtained from leaf, stem bark and root chloroform, ethyl acetate and methanolic extracts of *A.indica* and *C. hirsutus*was 3 g, 4 g, 5 g, 2 g, 2 g, 4 g, 1.5 g, 1.7 g and 2 g respectively out of 100 g of leaf, 250 g stem bark and 350 g root powders. More yield was obtained from leaf methanolic extract (5%) of *A. indica* (Table 1).

Phytochemical screening of the leaf, stem bark and root chloroform, ethyl acetate and methanolic extracts of *A. indica* have shown the presence of various medicinally active constituents (Table 2). A total of 11 phytochemicals were analysed. The leaf chloroform extract of *A. indica* showed the presence of six phytochemicals, leaf ethyl acetate extract showed two compounds and leaf methanol extract showed the presence of four compounds. The stem bark chloroform extract showed the presence of six phytochemicals, stem bark methanolic extract showed the presence of two compounds and ethyl acetate stem bark extract showed the presence of only one compound. The root chloroform extract showed the presence of three phytochemicals, root ethyl acetate extract showed the presence of three compounds and root methanolic extract showed the presence of three compounds. Phenolic compounds and glycosides were present in all the leaf extracts. Coumarins and quinones were present in all root extracts and phenolic compounds were present in all leaf extracts of *A. indica*.

The yield of extract obtained from leaf, stem bark and root chloroform, ethyl acetate and methanolic extracts of *C. hirsutus* was 2.5 g, 2.9 g, 11 g, 1.3 g, 1.7 g, 11 g, 1.5 g, 2.0 g and 10 g respectively out of 150 g of leaf, 170 g of stem bark and 250 g of root powders. More yield was obtained from leaf methanolic extract (5%) of *C. hirsutus* (Table 3).

Phytochemical screening of the leaf,

stem bark and root chloroform, ethyl acetate and ethanolic extracts of *C. hirsutus* showed the presence of various medicinally active constituents (Table 4). A total of 11 phytochemicals were analysed. The leaf chloroform extract of *C. hirsutus* showed the least number of compounds, leaf ethyl acetate extract showed the presence of three compounds, leaf methanol extract showed the presence of four phytochemicals. The stembark chloroform extract showed the presence of two compounds, stem bark ethyl acetate extract showed the presence of two compounds and stem bark methanol extract showed the presence of three phytochemicals. The root chloroform extract showed the presence of three compounds, root ethyl acetate extract showed the two compounds and root methanol extract showed the presence of four phytochemicals. Phenolic compounds were present in all leaf and stembark extracts. Alkaloids were present in all stem bark and root extracts. Phenolic compounds were present in all leaf and stembark extracts. Quinones and Glycosides were present in all leaf and root extracts.

In the present study, the plant parts of *A. indica* i.e., leaf, stem bark and root chloroform, ethyl acetate and methanol extracts of *A. indica* were evaluated for their antimalarial potencies against *P. falciparum* CQ-sensitive 3D7 and CQ-resistant K1 strains.The $IC_{50}$and CC_{50}values extracts of were represented in Table 5.

The leaf chloroform, ethyl acetate and methanol extracts of *A. indica* showed inhibitory concentrations of 3.34 µg/mL, 3.71 µg/mL and 32.85 µg/mL respectively; the stem bark chloroform, ethyl acetate and methanol extracts showed the inhibitory concentrations of 14.75 µg/mL, 19.07 µg/mL and 14.07 µg/mL respectively; the root chloroform, ethyl acetate and methanol extracts showed the inhibitory concentrations of 19.44 µg/mL, 23.54 µg/mL and 39.21 µg/mL respectively against *P. f.* CQ-sensitive 3D7 strain (Table 5).

The chloroform leaf, ethyl acetate leaf and methanol leaf extracts of *A. indica* showed inhibitory concentrations of 1.47 µg/mL, 2.32 µg/mL and 23.91 µg/mL respectively; the chloroform stem bark, ethyl acetate stem bark and methanol stem bark extracts showed inhibitory concentrations 12.25 µg/mL, 10.63 µg/mL and 7.92 µg/mL respectively; the root chloroform, ethyl acetate and methanol extracts showed inhibitory concentrations of 12.13 µg/mL, 9.64 µg/mL and 15.69 µg/mL respectively against *P. f.* CQ-resistant K1 strain (Table 5).

Thus, the above results revealed that, the leaf chloroform and ethyl acetate extracts of *A. indica* have shown IC_{50} values of <5 µg/mL which indicate their excellent antimalarial activity against 3D7 and K1 strains of *P. falciparum*.

The *in vitro*cyoto toxicity studies against Vero cell line were conducted for all the extracts of *A. indica*. All extracts showed CC_{50} value of >300 µg/mL. An extract is classified as a nontoxic when the CC_{50} value is >20 µg/mL.Based on this, all the extracts were not harmful for *in vivo s*tudies (Table 5).

In the present study, the plant parts of *C. hirsutus* i.e., leaves, stem bark and root extracted in chloroform, ethyl acetate and methanol were evaluated for their antimalarial potencies against *P. falciparum* CQ-sensitive 3D7 and CQ-resistant K1 strains.The $IC_{50}$and CC_{50}values extracts of *C. hirsutus* were represented in Table 6.

The leaf chloroform, ethyl acetate and methanol extracts of *C. hirsutus* showed inhibitory concentrations of 16.74 µg/mL, 15.47 µg/mL and >50 µg/mL respectively; the stem bark chloroform, ethyl acetate and methanol extracts showed inhibitory concentrations of 9.28 µg/mL, 8.10 µg/mL and 15.41 µg/mL respectively; the root chloroform, ethyl acetate and methanol extracts showed inhibitory concentrations of <0.78 µg/mL, 7.33 µg/mL and 3.34 µg/mL respectively against *P. f.* CQ-sensitive 3D7 strain (Table 6).

The leaf chloroform, ethyl acetate and methanol extracts of *C. hirsutus* showed inhibitory concentrations of 5.08 µg/mL, 6.81 µg/mL and 38.19 µg/mL respectively; the stem bark chloroform, ethyl acetate and methanol extracts showed inhibitory concentrations of 6.33 µg/mL, 4.54 µg/mL and 12.32 µg/mL respectively; the root chloroform, ethyl acetate and methanol extracts showed inhibitory concentrations of <0.78 µg/mL, 4.72 µg/mL and 2.10 µg/mL respectively against *P. f.* CQ-resistant K1 strain (Table 6).

Thus, the above results revealed that, the stem bark ethyl acetate extract against K1 strain, root chloroform extract against 3D7 and K1 strains, root ethyl acetate extract against K1 strain, root methanolic extract against 3D7 and K1 strains

of *P. falciparum* of *C. hirsutus* have shown IC_{50} values of <5 µg/mL which indicate their excellent antimalarial activity.

The *in vitro*cyoto toxicity studies against Vero cell line were conducted for all the extracts of *C. hirsutus*. All extracts showed CC_{50} value >300 µg/mL. An extract is classified as a nontoxic when the CC_{50} value is >20 µg/mL.Based on this all the extracts were not harmful for *in vivo s*tudies (Table 6).

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 (WHO), medicinal plants would be the best source to obtain a variety of drugs.

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 *A. 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*. who reported that the Hydro-alcoholic crude extract of *A. indica* showed positive test for flavonoids, steroids, tannins, amino acids and oils. The methanolic fraction of *A. indica* showed 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.[24]

The present work is supported by Kumarasamyraja *et al*. who screened 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. Ethylacetate extract of leaf showed only flavonoids. Methanol extract of leaf has shown tannins, phenols and saponins which is in correlation with our study.[25]

Prasad and Estari 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 result for 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.[26]

Similar work was done by Kumar *et al.* 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 saponinns 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*.[27]

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*. who reported the phytochemical analysis of different solvents of *C. hirsutus* plant. Petroleum ether showed positive test for oils and fats. Chloroform extracts 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.[28]

Meena also did phytochemical investigation of leaf methanolic extract of *C. hirsutus*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 extractions of *C. hirsutus*. Thus our study is in correlation with the peer researchers and our results confirm the earlier work.[29] Hence, the plants *A. indica* and *C. hirsutus* have various secondary metabolites which indicate the therapeutic potential for different ailments.

According to Inbaneson (2012), the stem ethanolic extract of *A. indica* showed good *in vitro* antimalarial activity (IC_{50} = 43.81 µg/mL) and leaf and root ethanolic extracts of *A. indica* showed IC_{50} values between (50-100 µg/mL) with mild activity.[30] But in our study, the leaf chloroform and ethyl acetate extracts of *A. indica* have shown IC_{50} values <5 µg/mL which indicate their excellent antimalarial activity against 3D7 and K1 strains of *P. falciparum*. Hence, it reveals that leaf extracts are more potent than stem and root extracts of *A. indica*. This might be due to the presence of many bioactive compounds such as alkaloids, saponins, terpenoids & steroids, tannins, phenolic compounds and glycosides in the leaf extracts of *A. indica.*[31]

Some other species belonging to the family Euphorbiaceae possess antimalarial activity. According to Udobang *et al*., the leaf extract of *Acalypha wilkensiana* dose-dependently reduced parasitaemia induced by CQ-sensitive *P. bergheii.*[32] Alshawsh *et al*. reported the excellent antiplasmodial activity of leaf aqueous extract of *A. fruticosa* IC_{50} value less than 4 µg/mL andleaf methanolic extract of *Acalypha fruticosa* showed antiplasmodial activity at IC_{50} value of 10.7 µg/mL against clinical isolates of *P. falciparum* and this might me due to the presence of tannins, terpenoids, flavonoids and polysaccharides.[33]

The plant *Cocculushirsutus*(L.) Diels belonging to the family Menispermaceae is a climbing scandent shrub with hairy sepals, used traditionally as alterative,laxative, demulcent, prurigo, eczema, dyspepsia tonic, diuretic, antiperiodic in fever, in malaria, joint pains and in skin diseases.Several phytoconstituents have been isolated like alkaloids, sterols and resins and identified from different parts of plant. Manystudies have been conducted to prove its potential as diuretic, laxative, antiinflammatory and antidiabetic properties.[34]

Elango *et al*. evaluated repellent, ovicidal and oviposition-deterrent potential of leaf hexane and chloroform extracts of *C. hirsutus* against *Anopheles subpictus* Grassi (Diptera: Culicidae) and caused a remarkable negative response resulting in oviposition of very few egg with deterrent effect.[35]The adulticidal activity and adult emergence inhibition (EI) of leaf hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. hirsutus* tested against *Anopheles subpictus* Grassi (Diptera: Culicidae). The report revealed that leaf methanol extract of *C. hirsutus* shown the highest adulticidal activity and has the potential to be used as an ideal eco-friendly approach for the control of *A. subpictus.*[36]

But the present study revealed the *in vitro* antimalarial activity of leaf, stem bark and root chloroform, ethyl acetate and methanol extracts of *C. hirsutus*. And the study reported that root chloroform extract of *C. hirsutus* has shown excellent antimalarial activity against 3D7 and KI strains of *P. falciparum*. Therefore this study provides the first report on the *in vitro* antimalarial activity of *C. hirsutus*againstCQ –sensitive (3D7) and CQ –resistant *P. falciparum*.

CONCLUSION

The present study revealed the presence of bio-active phytochemical constituents in plants *A. indica* and *C. hirsutus* which are responsible for the antimalarial activity. It concludes that the leaf chloroform and ethyl acetate extracts of *A. indica* and root chloroform extracts of *C. hirsutus* have shown excellent *in vitro* antimalarial activity against CQ-sensitive 3D7 strain and CQ-resistant K1 strains of *P. falciparum*. Further evaluation of the extract may provide potential molecule for therapy of malaria.

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