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Research Article

Analysis of Phytochemicals and Evaluation of Phenolic Contents and Antioxidant Activities of *Cissus javana* DC.

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ABSTRACT

Cissus javana belongs to the family Vitaceae growing in tropical forests was collected from the Western Ghats of Karnataka and subjected to qualitative phytochemical analysis, total phenolic content, and antioxidant activity. In the present study, different parts like the fruit stem and leaves of *C. javana* were subjected to preliminary phytochemical analysis in different solvent extracts, which revealed the presence of reducing compounds, alkaloids, flavonoids, tannins, sterols, terpenoids, glycosides, carbohydrates, resins, phenol, and proteins. The methanolic fruit extract of the sample showed the presence of most of the phytochemicals when compared to leaves and stem extracts of different solvents. The methanolic fruit extract showed a total phenolic content of about 2.94 mg of GAE, followed by leaves and stem 2.57 and 2.01 mg GAE/g, respectively. Antioxidant activities of methanol extract showed dose-dependent activity, which increased with an increase in the concentration of the extract. Methanol fruit extract showed the highest free radical scavenging activity, almost equal to the standard with the IC_{50} value of 4.925, where the IC_{50} value of ascorbic acid was found to be 3.389. The present investigation provides insights into the phytoconstituents and antioxidant activities of fruit stem and leaf extracts of *C. javana,* so it can be further subjected to purification of compounds that may act as an alternative for the current synthetic compounds that are used as pharmaceuticals.

Keywords: *Cissus javana,* Total phenol, Antioxidant, Phytochemicals

INTRODUCTION

Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grow wild in different parts of our country. India is rich in all three levels of biodiversity, as species diversity, genetic diversity and habitat diversity. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. Plants have been important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80% of people still rely mainly on traditional medicines. It has a vast traditional role in indigenous system of medicine like Ayurveda, Siddha, Unani and homoeopathy. India has very vast resource of indigenous plants and minerals which are an excellent source of therapeutic claim. All ethnic groups have been used plants as a source of medicines. Traditional medicines are still an incredible part of primary healthcare in the developing countries. In the developed countries too, people are turning towards herbal treatment because of its low side effect as compared to synthetic drugs. The Western Ghats, considered to be the one of the hotspots of biodiversity, supports an enormous vegetal wealth, covering the states of Goa, Maharashtra, Karnataka, Tamil Nadu and Kerala.[1]

Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compound. Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems. It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants.

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. People take alternative or complementary medicines mainly to avoid the side effects, and some others who have tried allopathic medicines but did not get relief from the ailment. So, there are interest indentifying plants or groups of plants that are used in traditional medicines around the world.[2] The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. Now a days we can extract the secondary metabolites from callus through 'Hairy root culture.[3]

Fig. 1: A: Habit of *Cissus javana* and B: flowers

The genus '*Cissus*' belongs to the Vitaceae family which includes the common fruit- grapes (*Vitis vinifera*) and possess several medicinal properties which were listed in Table 1. In recent years, plants of the genus *Cissus* were reported to contain sterols, triterpenoids, phenolics, flavonoids, stilbene derivatives, coumarin glycosides and iridous. Many *Cissus* species were reported to possess antimicrobial, anti-osteoporotic, hypoglycaemic, antioxidant, antitumor, analgesic, anti-inflammatory, gastro protective, hepatoprotective, immunomodulatory and anti-allergic activities.[4]

Cissus javana herbaceous climbing plant, climber over other plant or spread over ground (Fig. 1A and B). It is weak perennial climber with woody base. Stems are prominently red and hairless, and tendrils are forked. Leaves are ovate, lance shaped, with heart shaped leaves with pointed tip, margins finely toothed or bristly. Leaves usually mottled with aluminium white above, and purple on underside. Flowers are yellowish a red sepals cup and stalks, in small compound umbels opposite to leaves. The aqueous boiled extracts of leaves of *C. Javana* are generally used in the traditional treatment for the dissolution and expulsion of stones in the kidney and its tract. The leaf decoction of the plant is also used in joint pains and healing of fracture of bones. The kidney stone case is a serious clinical condition which may leads to major causes for acute and chronic renal failure.[5]

MATERIALS AND METHODS

Collection of Plant Materials

The fresh plant material of *Cissus javana* (Vitaceae) was collected from Nemmar region of Sringeri taluk, Chikamangaluru district, Karnataka. Western Ghats of Karnataka, India. The plant was identified with the help of Flora of Presidency of Madras. Fresh and tender leaves, stem and fruits of selected plants were used for phytochemical analysis.

Preparation of the Extracts

Different parts of *Cissus javana Viz*. leaves, stem and fruits were washed under running tap water, shade dried and powdered using mechanical blender. 50gm of dried leaves, stem and fruits powder was filled in the thimble and successfully extracted with petroleum ether; chloroform; ethyl acetate and methanol using Soxhlet extractor. All the extracts collected were concentrated using rotary flash evaporator and stored at 4°C in air tight containers used further studies.[18]

Phytochemical Test

The plant extracts were subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents like sterols, triterpenes, saponins, alkaloids, tannis, flavonoids, carbohydrates, resins, phenols, glycosides, proteins and terpinoids following the standard methods described by.[18,19]

Test for Sterols and Triterpenes

Salkowski test

Different solvent extracts when mixed with concentrated sulphuric acid and on standing results in formation of red colour specifying the presence of steroids.

Liebermann-burchard's test

To the extracts with a few drops of acetic anhydride and 1mL of con. Sulphuric acid was added along the sides of the test tube gives a reddish ring at the junction of two layers showing the presence of steroids.

Table 1: List of different species of *Cissus* genus used in medicine

S. No.	*Plant name*	*Location*	*Medicinal uses*	*References*
1	*C. araloides*	Cameroon	Anti-microbial	6
2	*C. assamica*	China, India, Cambodia, Bhutan, Nepal, Thailand	Anti-snake venom	7
3	*C. debilis*		Anti-cell proliferation	8
4	*C. hemaderohensis*	Yemen	Anti-viral, anti ACE, NGP and APN	9
5	*C. hypoplasia*	Australia	Sore-throat	10
6	*C. ibuensis*	Nigeria(Africa)	Rheumatism, arthritis, Gastrointestinal tract	11
7	*C. populnea*	Nigeria(Africa), Niger, Ghana	Increase proliferation of sertoli cells	12
8	*C. quadrangularis*	India, Sri Lanka(Asia)	Fracture healing increases bone strength, protects bone from postmenopausal bone loss	13
9	*C. rotundifolia*	Africa, South America	Anti-parasitic, anti-diabetic	14
10	*C. rubiginosa*	Congo	Anti-dysentery, anti-diarrhea	15
11	*C. sicyoides*	Brazil (South America)	Anti-diabetic, diuretic, anti-inflammatory, anticonvulsant, Anxiolyte	16
12	*C. verticillata*	Trinidad and Tobago(Carribean)	Anti-cholesterol, anti-diabetic	17

Detection of Saponins

Foam test

Extracts were diluted with distilled water to 20 mL and this was shaken well in a graduated cylinder for about 15-20 minutes. The formation of a foam layer over the solution indicates the presence of saponins.

Test for Alkaloids

Mayer's test

The plant extracts filtrates were treated with potassium mercuric iodide results in the formation of a creamy white colored precipitate indicates the presence of alkaloids.

Wagner's test

Few drops of Wagner reagent were added to the filtrate, formation of a brown-colored precipitate indicates the presence of alkaloids.

Dragendorff's test

The filtrates were treated with potassium bismuth iodide and the formation of an orange-colored precipitate indicates the presence of alkaloids.

Detection of Tannins

Ferric chloride test

One mL of the extract will be stirred well with one mL of ferric chloride solution, formation of greenish-black precipitate, indicates the presence of tannins.

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins.

Detection of Flavonoids

Shinoda test

To the extract add little magnesium turning followed by drop wise addition of con. HCL, the formation of crimson red or rarely green to blue colour appearance indicates the presence of flavonoids.

Ferric chloride test

Extracts were treated with 3 to 4 drops of ferric chloride solution. The formation of bluish-black colour indicates the presence of phenols.

Lead acetate test

Extracts were treated with a few drops of Lead acetate solution. The formation of a yellow colour precipitate indicates the presence of flavonoids.

Detection of Proteins

Biuret test

The extracts were treated with 1mL of 10% sodium hydroxide solution and heated. To this, a drop of 0.7% copper solution was added. The appearance of purplish violet colour indicated the presence of proteins.

Ninhydrin test

To the plant extract, 0.25% ninhydrin reagent was added and boiled for a few points. Appearance of blue colour showed the presence of amino acids.

Test for Carbohydrates

Molisch's test

The filtrate was treated with Molisch's reagent and a few drops of conc. Sulphuric acid was added along the sides of the test tubes. The formation of the violet ring at the junction of the liquid designates the presence of carbohydrates.

Fehling's solution

The filtrate was treated with a few mL of dilute HCL and heated in a water bath for 30min. for hydrolysis. Following that it was neutralized with sodium hydroxide solution. To the neutralized solutions, equal amount of Fehling's A and B solutions were added and heated in a water bath for a few minutes. The formation of a red-orange precipitate indicates the presence of reducing sugars.

Benedict's test

To the extract add 1-mL of Benedict's reagent and place in a water bath for 10 minutes. The appearance of brick red precipitate from the blue colour indicates the presence of reducing sugars.

Test for Resins

Acetic anhydride test

To the 1-mL of extract few drops of acetic anhydride solution was added followed by 1-mL of the con. Sulphuric acid was added, the formation of yellowish colour indicates the presence of resins.

Table 2: DPPH scavenging activity of methanol extracts of *C. javana*

Plant extracts	*Concentration (µg/mL)*					*IC_{50} value*
	20	40	60	80	100	
Leaf	DPPH % Scavenging					3.924
	15.60	24.54	34.58	56.87	61.25	
Stem	20.22	28.68	36.66	45.67	53.98	4.533
Fruit	14.88	21.22	31.23	41.23	51.55	4.925
Ascorbic acid	28.77	35.33	40.98	60.19	65.55	3.389

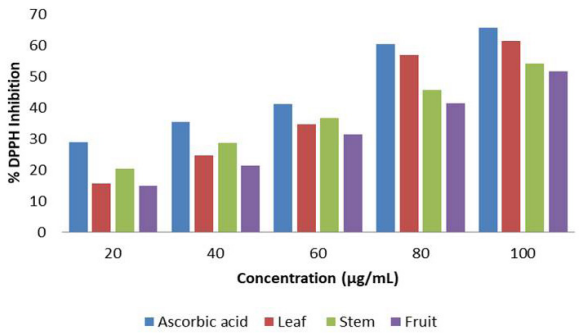

Fig. 2: DPPH scavenging activity of methanol extract of *C. javana*

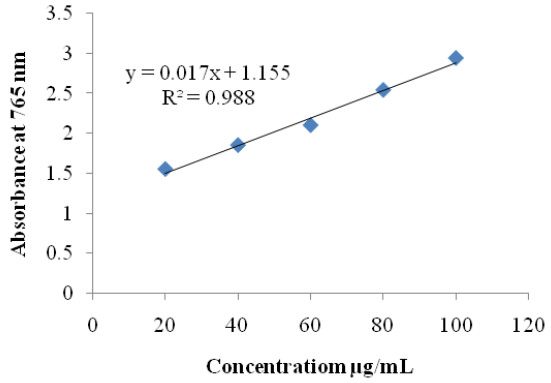

Fig. 3: Standard calibration curve for total phenolic contents

Detection of Cardiac Glycosides

Keller Killian test

The extract was diluted with 20 mL of distilled water; 1-mL of strong lead acetate solution was added to precipitate pigments, which are filtered off. The filtrate gained was shaken with an alike volume of chloroform and permissible to separate into two layers in a separating funnel. The chloroform layer was removed and evaporated to dryness over a water bath. The residue was dissolved in 3mL of ferric chloride in glacial acetic acid and then transferred to a dry test tube. A few drops of con. Sulphuric acid was added to the through along the sides of the test tube. On standing, a brown colour at the edge and a pale green in the upper layer designates the occurrence of cardiac glycosides.

Table 3: Phytochemical constituents of *C. javana* fruit, leaf and stem extracts

Tests	*Types*	*Fruit*				*Leaf*				*Stem*			
		PE	*CHL*	*EA*	*M*	*PE*	*CHL*	*EA*	*M*	*PE*	*CHL*	*EA*	*M*
Test for sterols	1.Salkowski test	–	–	–	+	–	–	–	+	–	–	–	+
	2. Liebermann Burchard's test	–	–	–	+	–	–	–	+	–	–	–	+
Test for triterpenes	1.Salkowski test	–	–	–	–	–	–	–	–	–	–	–	–
	2. Liebermann Burchard's test	–	–	–	+	–	–	–	+	–	–	–	+
Test for saponins	1. Foam test	–	–	–	+	–	–	–	+	–	–	–	+
Test for alkaloids	1. Mayer's test	–	–	–	–	–	–	–	–	–	–	–	–
	2. Dragendroff's test	–	–	–	–	–	–	–	–	–	–	–	–
	3. Wagner's test	–	–	–	–	–	–	–	+	–	–	–	–
Test for tannins	1. Ferric chloride test	–	–	–	–	–	–	–	–	–	–	–	–
	2. Gelatin test	–	–	–	+	–	–	–	–	–	–	–	+
Test for flavonoids	1. Shinoda test	–	–	–	+	–	–	–	–	–	–	–	–
	2. Ferric chloride test	–	–	–	–	–	–	–	–	–	–	–	–
	3. Lead acetate test	–	–	–	–	–	–	–	–	–	–	–	–
Test for carbohydrates	1. Fehling's test	–	+	–	+	–	+	–	–	–	+	–	–
	2. Benedict's test	–	–	–	–	–	–	–	–	–	–	–	–
	3. Molisch test	–	–	–	+	–	–	–	–	–	–	–	–
	4. Schiff test	–	–	–	+	–	–	–	–	–	–	–	–
Test for resins	1. Turbidity test	–	–	–	–	–	–	–	–	–	–	+	–
	2. Acetic anhydride test	–	–	–	+	–	–	–	–	–	–	–	–
Test for phenols	1. Phenol test	–	–	–	–	–	–	–	–	–	–	–	–
	2. Ellagic acid test	–	–	–	–	–	–	–	–	–	–	–	–
Test for glycosides	1. Keller-Kilani test	–	–	–	+	–	–	–	+	–	–	–	–
Test for proteins	1. Biuret test	–	–	–	–	–	–	–	–	–	–	–	–
	2. Ninhydrin test	–	–	–	–	–	–	–	–	–	–	–	–
Test for terpenoids	1. Terpenoids test	–	–	–	–	–	–	–	–	–	–	–	–

("+" indicates the presence of compounds and "-" indicates the absence of compounds)

Determination of Total Phenolic Content

Total phenolic compounds of the plant extracts were determined by following the method of[20] with minor modifications. Stock solutions of fruits, stem and leaves with the concentration of 1-mg-mL was prepared using the methanol extracts. In brief 50µL of plant extract was mixed with 950 µL of methanol such that volume was made up to 1-mL. 500 µL of 1:10 Folin-Ciocalteau reagent was added followed by 10% sodium carbonate solution. The blank consisted of 1mL of methanol. Gallic acid (ranging from 50–300 µL) was used as standard reference. The total phenolic content was determined as mg of gallic acid equivalent using an equation obtained from the standard gallic acid graph.

DPPH Assay

DPPH free radical scavenging activity was performed as described by[21] with few modifications in the standard protocol. The DPPH solution was prepared by dissolving 4 mg of standard DPPH in 100 mL of methanol. Briefly 20, 40, 60, 80, 100 µL of fruits stem and leaves extracts were taken from the stock solution (1-mg/mL) in different test tubes and made upto the 1-mL using methanol. Similar concentrations of ascorbic acid (1-mg/mL) was taken as standard. To each test tube 3 mL of DPPH solution was added. DPPH and absolute methanol was used for reagent blank. The mixture was vortexed for 1-minute, kept for 30 minutes in dark and then the absorbance was measured at 517 nm using spectrophotometer. The antioxidant activity of each sample was expressed in terms of IC_{50}, which was calculated from the graph after plotting inhibition percentage against concentration (Fig. 2 and Table 2). The percentages of the DPPH free radical scavenging activity were calculated as follows

$$\% \text{ scavenging activity } = \frac{(\text{Abscontrol} - \text{Abssample})}{\text{Abscontrol}} \times 100$$

RESULTS

Phytochemical Studies

The present study of phytochemical screening of crude extracts of leaf, stem and fruit of *C. javana* in different solvents like petroleum ether, chloroform, ethyl acetate, methanol was carried out using standard protocols. This qualitative analysis confirms the presence of various phytochemical constituents and the results are summarized in the Table 3. Methanolic leaf, stem and fruit extracts showed the presence of various phytoconstituents whereas, petroleum ether and ethyl acetate extracts showed minimum results. The chloroform leaf extracts of *C. javana* showed the presences of carbohydrates while the methanol leaf extracts showed the presence of sterols, triterpenes, saponins, alkaloids and glycosides. The chloroform stem extracts showed the presence of carbohydrates and ethyl acetate stem extracts showed the presence of resins. Carbohydrates, sterols, triterpenes, saponins and tannins were reported in the methanol stem extracts. The chloroform fruit extracts showed the presence of carbohydrates, whereas the methanol fruit extracts showed the presence of sterols, triterpenes, saponins, tannins, flavonoids, resins, glycoside and carbohydrates.

Antioxidant Activity

The results revealed that methanol extract of leaves showed excellent antioxidant activity when compared to methanol extracts of stem and fruit. There was complete discolouration of purple coloured solution to yellow colour in all the three extracts. As DPPH is relatively stable nitrogen centered free radical it can accept electron of hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agent as a result of which electron becomes paired off forming a corresponding hydrazine. The solution therefore loses colour stoichiometrically depending on the number of electrons consumed. There is also a slight discoloration of the solution in lower concentrations compared to complete discolouration in higher concentrations. This indicates that the antioxidant activity increases with the increase in the concentration. Methanol extract of leaves showed highest free radical scavenging activity which was almost equal to the standard with the IC_{50} value of 4.925 where the IC_{50} value of ascorbic acid was found to be 3.389 (Fig. 1).

Determination of Total Phenolic contents:

Phenolics or polyphenols are plant secondary metabolites and are very important by virtue of their antioxidant activity by chelating redox active active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxy radicals. The present study was carried out for the determination of total phenolic contents in leaf, stem and flowers of *Tithonia diversifolia* using methanol extracts. A total phenolics concentration equivalent of gallic acid was estimated according to Folin-Ciocalteau method. Gallic acid being one of the most important polyphenol in natural products, was used to determine the phenolics of tested plant extracts. The phenolic content of plant extracts was calculated by gallic acid equivalent. It is expressed as mg GAE/g (dry weight). The highest amount of phenolic content was found in leaf (2.94 mg GAE/g), followed by the stem (2.57 mg GAE/g) and flower extracts (2.01 mg GAE/g) respectively. The results obtained have considerable value and the activity of this extract may be attributed to the phenolic contents (Fig .3).

Antioxidant Activity by DPPH Assay

DPPH (2,2-diphenyl picryl hydrazyl) is a commercially available stable free radical, which is purple in colour. The antioxidant molecules present in the herbal extracts, when incubated, react with DPPH and convert it into di-phenyl hydrazine, which is yellow in color. The degree of discolouration from purple to yellow was measured at 517 nm, which is a measure of scavenging potential of plant extracts. Methanol extracts of leaf, stem and flowers were examined for free radical scavenging and the results were compared with standard antioxidant Ascorbic acid.

The results revealed that methanol extract of leaf showed excellent antioxidant activity when compared to methanol extracts of stem and fruit. There was complete discoloration of purple coloured solution to yellow colour in all the three extracts. As DPPH is relatively stable nitrogen centered free radical it can accept electron of hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agent as a result of which electron becomes paired off forming a corresponding hydrazine. The solution therefore loses colour stoichiometrically depending on the number of electrons consumed. There is also a slight discoloration of the

solution in lower concentrations compared to complete discolouration in higher concentrations. This indicates that the antioxidant activity increases with the increase in the concentration. Methanol extract of leaf showed highest free radical scavenging activity which was almost equal to the standard with the IC_{50} value of 4.925 where the IC_{50} value of Ascorbic acid was found to be 3.389 .

DISCUSSION

Medicinal plants are one of the main source for new pharmaceutical and health care products as most of the plants contain phytochemicals which has curetive/protective properties against various diseases. Most phytochemicals, especially phenolics have been proved to benefit health of the human beings by scavenging free radicals or quenching reactive oxigen species. Phenolics are, at least in the part, plants responsible for antioxidant activity, and their contents in the plants were associated with antioxidant activity. Ascorbic acid also has antioxidant activityand is essential for the maintainence of normal function of the living cells. It has reported that the majority of drugs from natural resources and that approximately 60-80% of the world's population still believe in folk/traditional medicine.[22]

Phytochemical analysis conducted on the plant extracts of *Cissus javana* revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The qualitative analysis carried out on leaf, stem and fruit extracts of the plant showed the presence of a good number of phytochemical constituents like triterpenes, carbohydrates, resins, tannins, flavonoids, saponins, glycosides, steroids, and alkaloids. The phytochemical analysis of stem and root of *Cissus populnea* showed the presence of alkaloids, flavonoids, saponins and tannins in large quantities. Finally concluded that *Cissus populnea* may serve as a potential source of useful drugs in future.[23]

A new alcohol was isolated from the aqueous methanol extract of the leaves of *Cissus javana* DC which is widely used in Manipur, India for dissolution and expulsion of kidney stones. In addition, known compounds Stigmasterol, Stigmasterol glucoside, Onocer-7-ene 3α,21β-diol, β-amyrin-[olean12(13)-en-3-one] were also isolated from the same methanol extract. Analysis of trace element contents of the plant leaf reflects its role in the ethno medicinal property of the plant.[24]

The medicinal properties of *Cissus auriculata* and *Cissus quadrangularis* along with pharmacological actions were studied by[25] These plants contain various active principles of therapeutic value and possess biological activity against number of diseases. In the present study free radical scavenging activity methanol extract of leaf showed highest activity which was almost equal to the standard with the IC_{50} value of 4.925 where the IC_{50} value of ascorbic acid was found to be 3.389. Antioxidant activity of leaf and stem extracts of *Cissus multistriata* was determined by DPPH method by spectrophotometrically. The highest radical scavenging effect was observed in the stem extract with IC_{50} of 29.25 µg/mL.[26] The ethanol extract of *Cissus quadrangularis* was used as anti-osteoporotic activity. It worked on healthy albino rats. So the ethanol extract of the *Cissus quadrangularis* showed definite anti-osteoporotic effect.[27]

CONCLUSION

The phytochemical analysis revealed that the plant contains triterpenes, carbohydrates, resins, tannins, flavonoids, saponins, glycosides, steroids, and alkaloids. The methanol extracts of leaves and stem showed excellent radical scavenging activity which was significantly comparable to free radical scavenging activity of Gallic acid. The findings of this study suggests that the leaves and stem of this plant could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the process of ageing and oxidative stress related degenerative diseases. The overall results explain the presence of several medicinally important constituents in *C. javana*, providing health application at affordable cost. However, further investigation, isolation and characterization are essential to draw decisions about the properties of the secondary metabolites and their potency. The study provides justification for the therapeutic use of these plants as natural antioxidants in folklore medicine.

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