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Research Article

PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS OF LEAF EXTRACTS FROM *KIRGANELIA RETICULATA* BAILL.

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ABSTRACT

In the present study we carried out a systematic record of the phytochemical and antioxidant properties of the medicinal plant *Kirganelia reticulata*. The different solvent extracts of *Kirganelia reticulata* leaves were screened for their *in vitro* phytochemical and antioxidant activity. Leaves were extracted with solvents of different polarities like aqueous, ethanol, methanol, chloroform, acetone and hexane. The distributions of the main active principles such as alkaloid, flavonoids, phenols, steroids tannins etc present in the plant were analyzed. It was also focused to determine the total phenolic and flavonoid content present in the extracts. Extracts showed promising results for total antioxidant capacity and reductive capability when compared with standard drug. The ethanol extract was found to possess excellent phytochemical and antioxidant activities. The antioxidant property may be attributed to the presence of flavonoids and phenolics present in the drug. The ability of the crude extracts of *Kirganelia reticulata* towards reduction, presence of phenol, flavonoid and antioxidant is an indication of its broad spectrum potential which may be employed in the management of various diseases.

Keywords: Antioxidant, *Kirganelia reticulata,* Phytochemical, Total phenolics, Total flavonoids

INTRODUCTION

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal plants". Medicinal plants are the local heritage with global importance. World is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to the development of ancient *materia medica*. Drugs of herbal origin have been used in traditional systems of medicine such as Unani and Ayurveda since ancient times[1]. The drugs are derived either from the whole plant or from different organs like leaves, stem, bark, root, flower, seed etc. These medicines are safer and environment friendly[2].

Kirganelia reticulata (Phyllanthus reticulatus) belongs to family Euphorbiaceae, grows through tropical areas of India, China, Bangladesh and Malay islands[3,4]. It is a monoecious scandent shrub present in hill areas and plain lands. The juice of these leaves is used to cure diarrhoea in infants. The leaves are employed as diuretic and cooling medicine. The stems are used to treat sore eyes and the powdered leaf is used in sores, burns, suppurations and chafing of the skin[5]. The bark is used to treat rheumatism, dysentery and veneral diseases[6]. The bark is also used for a variety of ailments including small pox, syphilis, asthma, diarrhoea, bleeding from gums[7]. The biological work performed so far on the plant showed hypotensive effects in gastric complaints including colic, constipation etc and chemical studies demonstrated the presence of octacosanol, texerol acetate, berulin, sitosterol etc[8,9]. It is believed to have antidiabetic activity in tribal areas[10]. The antibacterial potential of the leaf extracts of this plant has been evaluated recently[11]. Pharmacognostic parameters like microscopy, quantitative leaf microscopy, fluorescence, physiochemical properties are studied. Different chemical compounds such as tannins, flavonoids, glycosides, and alkaloids serves as a sink for several bioactive compounds[12].

Phytochemicals are chemical compounds, occur naturally in plants. Phytochemicals have been used as drugs for millennia. Phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects[13]. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Antioxidants are widely used as ingredients in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. These compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline. Flavonoids are also found to be powerful anti-oxidants and researchers are looking into their ability to prevent cancer and cardiovascular diseases.

So the present study is focused towards the identification of secondary metabolites present, estimation of flavonoids, phenolics and antioxidants present in the plant. Hence, an effort has been made to explore the above properties of herbal extracts which proves the potency of the plant as a source of natural antioxidants or nutraceuticals with application to reduce oxidative stress with consequent health benefits.

MATERIALS AND METHODS

Fresh leaf materials of *Kirganelia reticulata* plant were collected in summer season in and around HSR layout, Bangalore, Karnataka (Southern India). The taxonomic identification of the plant was confirmed and processed for further investigations.

Extraction of plant material

The collected leaves were washed thoroughly with distilled water. Cleaned leaves were then air-dried in shade at room temperature (26°C) for 2 weeks, after which it was grinded to uniform powder. About 1gm of dry powdered plant materials were soaked in 10 ml of aqueous, ethanol, methanol, chloroform, acetone and hexane (LR grade, Merck, India) at room temperature for 48 h. The extracts were filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using rotary evaporator (Buchi Flawil, Switzerland). The percentage yield of extracts ranged from 7–19% (w/w).

Phytochemical screening

Phytochemical screenings were performed for carbohydrates, alkaloids, phytosterols, glycosides, deoxysugars, saponins, phenolics, tannins, flavonoids and gum using standard procedures[13,14].

Carbohydrates: The presence of carbohydrates was determined by Benedict's method.

Alkaloids: Mayer's test was performed to test alkaloids

Phytosterols: Phytosterols presence was tested by Salkowski's method

Glycosides: The presence of glycosides was determined by Legal's test.

Deoxy sugars: Keller–Kiliani test was used for determination of Deoxy sugars

Saponins: Saponins were determined by Froth method

Phenolics and tannins: Ferric chloride test was conducted to determine the presence of it.

Flavonoids: Lead acetate test was used for flavonoids.

Gums test: Presence of gum was determined by Borax method

Determination of total phenolics

The total phenolic content in the extract was determined with Folin-Ciocalteu's reagent (FCR). 0.5ml of extract was mixed with 2ml of FC reagent and 2ml of sodium carbonate. The tubes are kept at room temperature for 90 mins. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using gallic acid as standard (20mg/100ml in distilled water, working standard taken as 10ml stock made upto 100ml with distilled water). The phenolic content was expressed in terms of milli grams of gallic acid[15].

Determination of total flavonoids

1ml of test sample was taken and 4ml of water was added. 0.3ml of Sodium nitrate, 0.3ml of aluminium chloride was added. After 6min incubation at room temperature, 2ml of NaOH was added to the reaction mixture, made the volume to 10ml by adding distilled water. The absorbance of the reaction mixture was measured at 510 nm against blank by using spectrophotometer. Catechin hydrate was used as standard (20mg/100ml in distilled water). Total flavonoids were expressed as catechin equivalents in milligrams.

Determination of total antioxidant capacity

The antioxidant activity of the extracts was determined by the phospho molybdenum method[16]. The 0.3ml of extract was combined with 3ml of reagent solution (0.6M sulphuric acid, 4mM ammonium molybdate, 28mM sodium phosphate). The reaction mixture was incubated at 95^0C for 90 min and cooled to room temperature. Absorbance of the solution was measured at 695nm using a spectrophotometer against blank. Ascorbic acid was used as standard (1mg/ml in distilled water); the total antioxidant capacity was expressed as the number of equivalents of ascorbic acid (AAE).

Total reductive capability (Fe^{3+}- Fe^{+2} transformation)

Different concentrations of extracts were mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6), 2.5ml of 1% potassium ferri cyanide and 2.5ml of 10% trichlroacetic acid, which is then centrifuged at 660rpm for 10 min. The upper layer of the solution was mixed with 2.5ml distilled water and 0.5ml of 0.1%$Fecl_3$. The amount of iron (II) ferri cyanide complex formed was determined by measuring absorbance of the reaction mixture at 700nm in spectrophotometer. Increased absorbance indicates increased reduction capability, Fe (III) reduction is often used as an indicator of electron donating activity. Quercetin was used as standard (25mg/ml in distilled water)[17].

Statistical analysis

All the experiments are carried out in triplicates. The values were expressed as mean ± SEM. Statistical analysis of data was performed using ANOVA followed by student t-test to study the differences amongst the means[18]. Values of $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The phytochemical analysis carried out on the plant revealed the presence of several medicinally active constituents. Ethanol extract has shown to contain large number of secondary groups such as carbohydrates, alkaloids, phenols, flavonoids, saponins, gum, glycosides etc. The results of which are shown in **table 1**. It may be attributed to the reason that the stronger extraction capacity of ethanol could have extracted a greater number of constituents. These compounds are known to be biologically active and hence aid the investigation of several activities. These observations therefore support the use of *K. reticulata* in herbal cure remedies. Alkaloids were detected together with flavonoids; this may be responsible for the antioxidant activity observed in the crude extracts. According to van Beek *et al*. [19], who studied a large number of plant extracts against various activities, ethanolic extracts always show positive effect. The preliminary phytochemical evaluation revealed the presence of several secondary metabolites which are known to possess various pharmacological effects. In last four decades the scientists are keen to evaluate many plant drugs used in medicinal folklore, due to their specific healing properties, health action and non toxic effects[20].

Although similar to alcohols, phenols have unique properties and are not classified as alcohols; since the hydroxyl group is not bonded to saturated carbon atom. They have higher acidities due to the aromatic ring's tight coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. Some phenols are germicidal and are used in formulating disinfectants where as others possess estrogenic or endocrine disrupting activity. So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts[21]. The content of total phenols in the extracts expressed in gallic acid equivalents (GAE) varied between 1.79±0.02, 1.69±0.03 and 0.59±0.02 mg/g in ethanol, acetone and hexane extracts respectively (**Fig 1**). The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties[22]. According to our study, the high phenolic content in ethanol extract can explain its high free radical scavenging activity.

Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities. They are also found to be powerful anti-oxidants and researchers are looking into their ability to prevent cancer and cardiovascular diseases. Flavonoids are most commonly known for their antioxidant activity.

Flavonoids acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine[23,24]. The quantitative determination of flavonoids in plant extracts shows that they are good source of flavonoids. High quantity of it was found to be 0.6±0.02 mg/g in ethanol extract which is followed by acetone 0.24±0.01, whereas in hexane extract it was recorded to have the least value of 0.19±0.01 mg/g (**Fig 2**).

A variety of intrinsic antioxidants (SOD, CAT, peroxidase, reduced gluthathione) are present in the organism, which protect them from oxidative stress, thereby forming the first line of defense[25]. Furthermore, the antioxidant activities of putative antioxidants have been attributed to various mechanisms; among these are prevention of chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging[26]. As stated before, numerous polyphenols are known to possess excellent antioxidant effects, especially *in vitro* and the amount of polyphenols present in a plant extracts has been suggested to correlate with the antioxidant activity. For example, Chinnici *et al.*[27] and Leontowicz *et al.* [28] found good correlations between total polyphenols and the total antioxidant activity. In our study, however, same correlation was found, showing that total antioxidant present in ethanol extract was high 0.54±0.01, followed by acetone 0.45±0.05 and hexane 0.42±0.03 extracts compared to standard as shown in **fig 3**.

The reducing power can serve as a significant reflection of the antioxidant activity. The reducing properties are generally associated with the presence of reductones. Gordon[29] reported that the antioxidant action of reductones is based on the breaking of the free radical chain by the donation of a hydrogen atom. Increasing absorbance at 700 nm indicates an increase in the reductive ability. Ethanol and acetone extracts showed highest reductive ability such as 0.43±0.04 and 0.32±0.03, compared to hexane extract 0.12±0.02 which was not active considerably. But all the extracts comparatively showed very lesser reductive activity than the standard Quercetin. The results of which are shown in **fig 4**.

However, more detailed analytical information on the constituents mediating the observed biological effects is needed prior the promotion or development of effective and safe foods for human consumption.

Table 1: Phytochemical investigations of *Kirganelia reticulata*

Secondary Metabolites	Extracts					
	Aqueous	Ethanol	Methanol	Chloroform	Acetone	Hexane
Carbohydrates	+	+	+	+	-	+
Alkaloids	+	+	+	+	+	+
Phytosterols	+	-	+	-	+	+
Deoxysugars	-	-	-	+	-	+
Saponin	+	+	-	-	-	-
Phenol	+	+	+	+	+	-
Tannin	-	-	-	+	-	-
Flavonoids	+	+	+	-	+	-
Proteins	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-
Gum	+	+	+	+	+	-
Glycosides	+	+	+	+	+	+
Antraquinone glycosides	-	+	+	+	+	+

'+'-present, '-'-absent

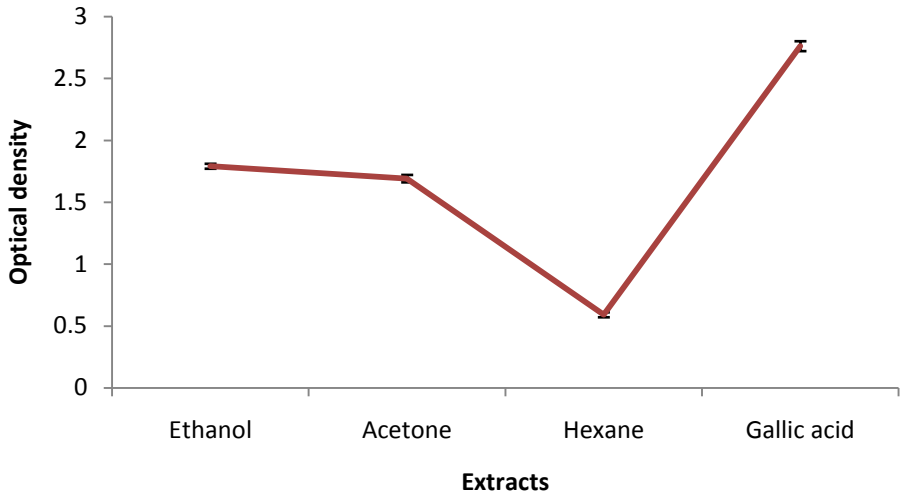

Fig. 1: Total phenolic content present in the leaf extracts of *Kirganelia reticulata.* Values are expressed in Mean ± SE, n=3.

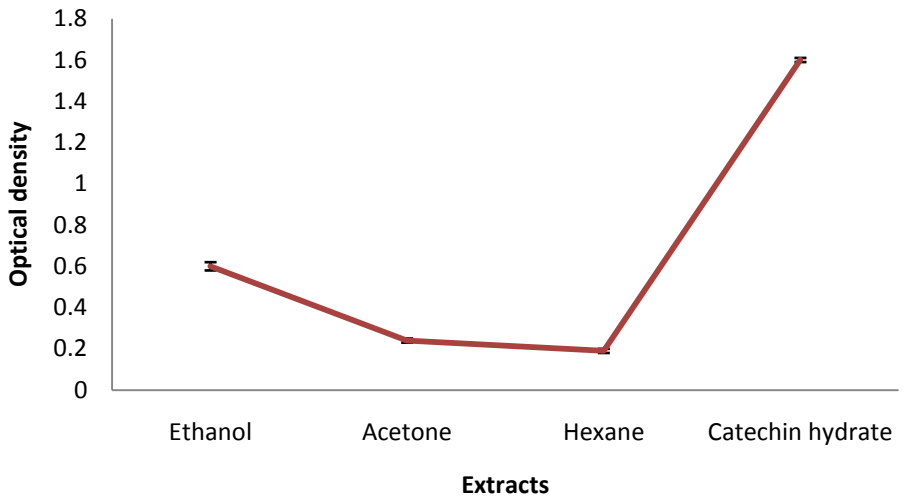

Fig. 2: Total flavonoid content present in the leaf extracts of *Kirganelia reticulata.* Values are expressed in Mean ± SE, n=3.

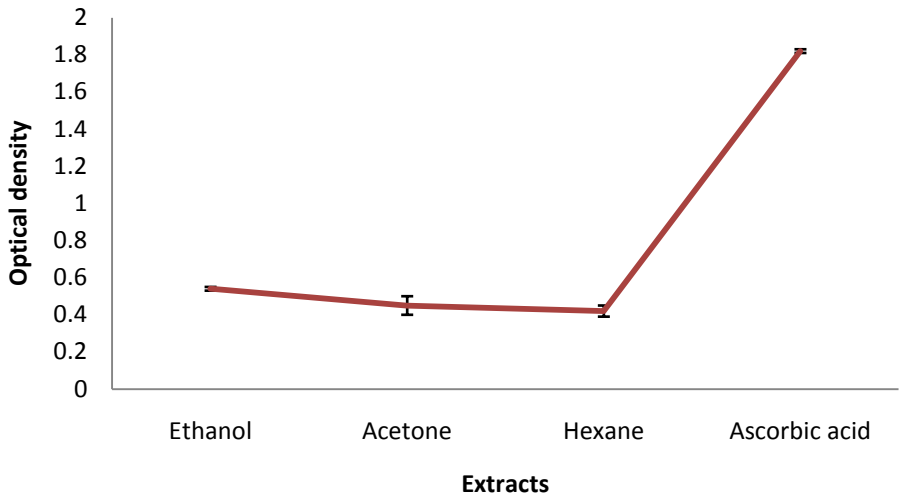

Fig. 3: Total antioxidants present in the leaf extracts of *Kirganelia reticulata.* Values are expressed in Mean ± SE, n=3.

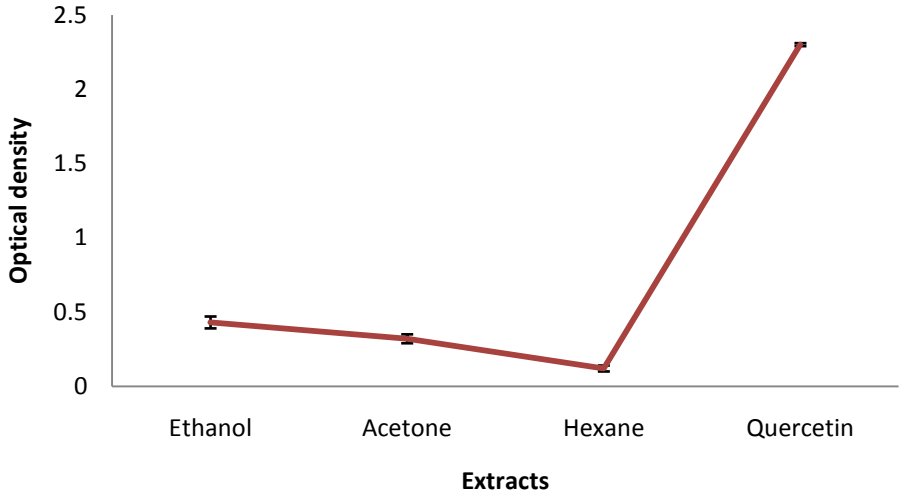

Fig. 4: Total reductive capability the leaf extracts of *Kirganelia reticulata.* Values are expressed in Mean ± SE, n=3.

CONCLUSION

The systemic research for useful bio actives from the plants is now considered to be a rational approach in nutraceuticals and drug research. The results of phytochemical analysis comprehensively validate the presence of therapeutically important and valuable secondary metabolites. Along with, it confirms the ethanobotanical claim of the plant to be a potential antioxidant. Hence, the plant contains good store of antioxidants and essential metabolites to support its efficiency to be a drug. It can be recommended as dietary supplement there by the nutritive potential indulged by the plant is yet to explore.

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