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Original Article

Phytochemical Analysis, Antioxidant Assay and Antimicrobial Activity in Leaf Extracts of *Cerbera odollam Gaertn*

Abinash Sahoo, Thankamani Marar*

ABSTRACT

Introduction: In the current study, methanol and aqueous extracts of leaf of *Cerbera odollam Gaertn* were screened for its antibacterial, antifungal, phytochemicals and antioxidant activities. Phytochemical constituents were investigated both qualitatively and quantitatively. **Methods:** The leaf extracts of *Cerbera odollam Gaertn* were prepared by drying and extracted using Soxhlet apparatus into methanol and aqueous media, which were subjected to phytochemical screening. Total phenols, tannins, flavanols, alkaloids and its antioxidant activity were determined using spectroscopic techniques. Antimicrobial activity were determined using well diffusion method. **Results:** Aqueous extract exhibits higher content of phenols, tannins, flavanols and alkaloids, whereas methanol extract exhibits higher content of anthocyanin and cardiac glycoside respectively. Aqueous extract exhibits higher inhibitory concentration (IC %) value for DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and H_2O_2 radical scavenging assay and reducing power (RP) assay. The methanol extracts exhibited higher inhibitory concentration (IC %) value in SO and NO radical scavenging assay, exhibiting antioxidant properties in five antioxidant models that were investigated. The methanol extract showed some antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* with inhibitory zone ranging from 2 mm to 3 mm, whereas the aqueous extract showed no activity. High antifungal activity was found against *Saccharomyces cerevisiae* and *Candida albicans* for methanol extract and moderate for aqueous extract with inhibitory zone ranging from 9mm to 26 mm**. Conclusion:** The finding of our study have suggested that the extracts of *Cerbera odollam Gaertn,* possesses a significant amount of phytochemicals and exhibits antioxidant and antifungal activities.

Key words: *Cerbera odollam. G*, Phytochemicals, Antioxidants, Radical scavenging, Antibacterial, Antifungal.

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INTRODUCTION

Cerbera odollam Gaertn is a tree belonging to the family of Apocynaceae and widely distributed on the coastal area of South East Asia and around the Indian Ocean. The fruits and seeds of *C. odollam* are highly toxic, containing cardiac glycoside like cerberin, neriifolin, odollin, etc. responsible for 10% to 50% of total poisoning cases in the state of Kerala, India. It is used widely for suicide and homicide and hence the name "suicide tree".[1] The tree is also known for various medicinal properties. The latex is known in India for its emetic, purgative and irritant effects.[2] The oil from the seeds as a cure for, itching or applied to the hair as an insecticide.[3] The bark and leaf of the plant are traditionally used as emetic and cathartic; kernels are used as an emetic; fruit is used as a cure for hydrophobia.[4] Its bark and fruits are purgative and used for the treatment of rheumatism.[5] There are reports of the anti-nociceptive and sedative effects of its barks.[6] Other research works have reported cytotoxic activity,[7] its effect on the central nervous system,[3] purgative and antirheumatic activity,[8] cardiac stimulant activity,[9] neurological activities,[10] and cardiotoxic activity.[11]

Medicinal properties of *C. odollam* are associated with various phytochemicals found in the plant. Different plant species have shown cytotoxic activity against bacteria, fungus, and virus. It also possesses the property to scavenge reactive oxygen species (ROS) which is the major source of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders[12,13] such as cancer[14] cardiovascular disease,[15] neural disorders,[16] Alzheimer's disease,[17] mild cognitive impairments,[18] Parkinson's disease,[19] ulcerative colitis,[20] ageing[21] and atherosclerosis.[22]

Phytochemicals are natural bioactive compounds widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radicals scavenging, anti-inflammatory, anti-cancer, antibacterial, antifungal etc. Therefore, the need arises to evaluate and quantify these phytochemical constituents.

The present study was designed to determine the phytochemical constituents, antioxidant activity,

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antibacterial and antifungal activity in methanol and aqueous extract from the leaves of *Cerbera odollam Gaertn* through a number of testing methods.

METHODS

Collection and extraction of plant material.

Fresh leaf of *Cerbera odollam Gaertn* collected from Mumbai, Maharashtra, India and authenticated by Blatter herbarium, St Xavier's college, matching with the Blatter herbarium specimen NI 2084 of N.A. Irani. Leaves were cleaned and dried at room temperature for a period of 25 days under shade. Finely ground dried leaf, were weighed and extracted using Soxhlet apparatus by two different solvents methanol and distilled water, 150 ml each for 30 g of powder. The solution of each extract was then subjected to rotatory evaporator and reduced to $1/8^{th}$ volume. The solution obtained was stored at 4°C and appropriately diluted for further studies.

Phytochemical screening (qualitative study)

Test for Alkaloids - Mayer's Test.[23]

Few mg of the residue of each extract was taken separately in 5 ml of 1.5 % v/v hydrochloric acid and filtered. These filtrates were treated with Mayer's reagent (1.36 g mercuric chloride and 5 g of potassium iodide dissolved in 100 ml distilled H_2O). The formation of a yellow cream precipitate indicates the presence of alkaloids.

Test for Flavonoids - Alkaline reagent test.[24]

Two ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Saponins - Foam test.[25]

About 2 ml of distilled water and 1 ml of extract were mixed and shaken vigorously. Formation of a stable persistent froth indicated the presence of saponins.

Test for Tannins - Potassium Dichromate Test.[26]

The test residue of each extract was taken separately in water, warmed and filtered. If on an addition of a solution of potassium dichromate in test filtrate, dark colour develops, tannins are present.

Test for Carbohydrates - Molisch's test.[23]

Few drops of Molisch's reagent were added to a 2ml portion of the extracts. This was followed by addition of 2ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 min. Formation of a red or dull violet color at the interphase of the two layers was a positive test for the presence of carbohydrates.

Test for Phenols - Ferric chloride test.[27]

One ml of the extracts in 3ml of distilled water was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color indicating presence of phenols.

Test for Cardiac glycosides - Keller Kelliani's test.[28]

Five ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar, characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Test for Amino acids -Ninhydrin test[29]

Two ml of the filtrate was treated with 2-5 drops of 1% ninhydrin solution placed in a boiling water bath for 1-2 min and observed for the formation of purple color.

Test for Sterols - Liebermann-Burchard test[28]

One ml of the extract was treated with drops of chloroform, acetic anhydride, and conc. H_2SO_4 and observed for the formation of dark pink or red color.

Test for Terpenoids - Salkowki's test[23]

One ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones[30]

To 1 ml of extract, 1 ml of conc. H_2SO_4 was added. Formation of red color indicated the presence of quinones.

Test for Oxalate[28]

To a 3ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates the presence of oxalate.

Test for Proteins. - Biuret Test[31]

Extracts were treated with 1 ml of 10% NaOH solution and heated. To this, a drop of 0.7% $CuSO_4$ solution was added. Formation of purplish violet color indicates the presence of proteins.

Test for Phlobatannins[32]

About 2 g of powdered sample was boiled with 1% aqueous hydrochloric acid for 5 min. A positive test result was confirmed by deposition of a red precipitate.

Test for Betacyanins[32]

To 2 ml of plant extract, 1 ml of 2N NaOH was added and heated for 5 min at 100^0 C. Formation of yellow color indicated the presence of betacyanin.

Test for Fatty acids[33]

Extract (0.5ml) was mixed with 5 ml of ether and allowed for evaporation, on filter paper and dried. The appearance of transparent spots filter paper indicates the presence of fatty acids.

Estimation of total phenols

The total phenol content was determined using the Folin-Coicalteu method.[34] Extract and 0.1ml of Folin Coicalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate (20%) was added and after 30 min absorbance measured at 760 nm. The total phenol content was expressed in terms of gallic acid equivalent (mg/g).[35]

Estimation of total tannins

The total tannins content was determined using Broadhurst and Jones (1978) method. HCl (8%) in methanol and 4% vanillin in methanol were added to the extracts and absorbance was measured at 500 nm against blank after 20 mins of incubation at room temperature. The total tannins content was expressed in terms of tannic acid equivalent (mg/g).[36]

Estimation of total flavonoids

The total flavonoids content was determined using aluminium chloride method.[37] The reaction mixture comprising of extract, aluminium chloride and potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoid content was expressed in terms of quercetin equivalent (mg/g).[38]

Estimation of total alkaloid

The total alkaloid content was determined using Dragendroff's sodium sulfide method. Mixture containing extract, Dragendroff's reagent and 1% sodium sulfide was centrifuged to collect the precipitates. It was dissolved in conc. nitric acid and 3% thiourea was added. The coloured solution was checked for the absorbance at 435 nm. The total alkaloid content was expressed in terms of boldine equivalent (mg/g).[39]

Estimation of total anthocyanin

The total anthocyanin content was determined using two buffer systems – potassium chloride buffer, pH-1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). Extract (0.4 ml) was mixed with 3.6ml of the corresponding buffer and the absorbance was measured against the blank at 510nm and 700 nm respectively in UV spectrophotometer for each extract, the difference between A_{510} and A_{700} for pH-1 and pH-4.5 individually was taken. Absorbance was calculated as: $A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5.}$ Monoamine anthocyanin pigment concentration in the extract was calculated as cyanidin-3-glycoside (mg/l) = A × MW × DF × 1000 / MA. Where A: absorbance; MW: molecular weight (449.2); DF: dilution factor; MA: molar absorptivity (26,900). The total anthocyanin content was expressed in terms of cyaniding-3-glycoside equivalent (mg/g).[40]

Estimation of cardiac glycosides

The total cardiac glycosides content was estimated according to Solich *et al,* by some modification. The extracts were filtered using Whatman paper no. 1. To each of the standard and extract solution, 1ml of Baljet's reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH) was added. After 1 h of incubation, the mixture was diluted with 20ml distilled water and absorbance was measured at 495 nm against a blank containing D/W. The total cardiac glycoside was expressed in terms of digoxin equivalent (mg/g).[41]

Evaluation of antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1- diphenyl-1-picryl-hydrazyl by the method of McCune and Johns.[42] The reaction mixture consisted of DPPH in methanol (0.3mM) and 1 ml of extract. After incubation for 10 min in dark, the absorbance was measured at 517 nm against methanol as blank. DPPH scavenging activity was expressed in terms of ascorbic acid equivalent,[35] as percentage inhibition calculated by the formula:

$$\%\ \text{inhibition of DPPH} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

H_2O_2 radical scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide is assessed according to the method of Ruch.[43] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4) and 2 ml of the solution was added to 1ml extract (1:20 dilutions). The absorbance at 230 nm was determined after 10 mins in dark, against phosphate buffer as blank. H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalent, as percentage inhibition calculated by the formula:

$$\%\ \text{inhibition of } H_2O_2 = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Super oxide radical scavenging assay

The superoxide radical scavenging activity was measured as described by Robak and Gryglewski.[44] The superoxide anion radicals are generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8), containing 0.5 ml of NBT (0.3 mM), 0.5ml NADH solution (0.936 mM) and 1.0 ml extract (1:100 dilution). The reaction was started by adding 0.5 ml PMS (0.12 mM) solution to the mixture, incubated at 25°C for 5 min and then the absorbance was measured at 560 nm against Tris-HCl buffer as blank. SO anion scavenging activity was expressed in terms of ascorbic acid equivalent,[35] as percentage of inhibition was calculated by the formula:

$$\%\ \text{inhibition of SO} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Nitric oxide radical scavenging assay

The nitric oxide radical scavenging activity was estimated using Griess's reaction as described by Chanda and Dave.[35] 3ml of sodium nitroprusside in phosphate buffer (10 mM) was added to 2 ml of extract (1:200 dilution). The resulting solution was then incubated at 25°C for 60 min. To 5ml of the incubated sample, 5ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) was added and the absorbance of the chromophore formed was measured at 540 nm against phosphate buffer as blank. NO radical scavenging activity was expressed in terms of ascorbic acid equivalent, as percentage of inhibition was calculated from given formula:

$$\%\ \text{inhibition of NO} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Reducing power assay

Reducing power assay was determined according to the method of Oyaizu.[45] To the extract, 2.5 ml of phosphate buffer was added. Then 2.5 ml of potassium ferricyanide was added and the tubes were incubated at 50°C for 20 min (in dark). Then 2.5 ml of TCA was added. The solution obtained was then centrifuged at 3000 rpm for 10 min. Ferric chloride solution (1ml) was added to the supernatant and the absorbance was read at 700 nm against phosphate buffer as blank. Reducing power was expressed in terms of ascorbic acid equivalent.[46]

Anti-bacterial assay

Anti-bacterial assay was determined using agar well diffusion method on nutrient agar plates with wells bored into them. Culture obtained was then spread across the agar and allowed to stand for 10 min, under sterile condition. 100μl of the extract was placed into the well with tetracycline as positive control and sterile distilled water as negative control. After diffusion of the extract, the plates were incubated 37°C for 32 hr. Zone of inhibition was measured.[47]

Three gram positive organisms and three gram negative organisms namely *Corynebacterium diphtheria, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia* were tested for antibacterial activity against the control tetracycline.

Anti-fungal assay

Anti-fungal activity was determined using agar well diffusion method on Sabouraud's agar plates with wells bored into them. Culture obtained was then spread across the agar and allowed to stand for 10 min, under sterile condition. 100μl of the extract was placed into the well with fluconazole as positive control and sterile distilled water as negative control. After diffusion of the extract, the plates were incubated 37°C for 32 hr. Zone of inhibition was measured.[47]

Two fungal species namely *Saccharomyces cerevisiae* and *Candida albicans* were used for studying antifungal activity against the control fluconazole.

RESULTS AND DISCUSSION

Qualitative screening of *C. odollam G*.

Results obtained from qualitative screening from leaf extracts of *C. odollam. G* is presented in Table 1. A total of 16 tests were carried out for detection of different phytochemicals. Of which 13 of them were present in both the extracts. These were alkaloids, flavonoids, tannins, carbohydrates, phenols, cardiac glycosides, amino acids, terpenoids, quinones, oxalates, proteins, fatty acids, and betacyanins.

The results indicate that *Cerbera odollam Gaertn* holds promise as a source of pharmaceutically important phytochemicals. Hence quantitative determination of these phytochemicals becomes crucial.

Total phenolic, tannin, flavonoid, alkaloid, anthocyanin, cardiac glycoside, chlorophyll contents and antioxidant activities of *Cerbera odollam G*.

The total phenolic content for dry weight of *Cerbera odollam G* was estimated to be 72.17 mg/g for methanolic extracts and 78.46 mg/g for aqueous extracts (Table 2). Phenols are reactive species towards oxidation and pose biological activity. The process of oxidation and free radicals generation leads to cancer and other diseases. The activity of phenols against this cancer causing process can have therapeutic application in anticancer therapies. Plants having more phenol content show good antioxidant activity indicating a direct correlation between TPC and antioxidant.[35]

The total tannins content of leaf extracts of *Cerbera odollam G was* estimated to be 44.69 mg/g dry weight for methanolic extracts and 90.99 mg/g dry weight for aqueous extracts (Table 2). Tannins are mostly found in stem and barks of many plants. High concentration of tannins in aqueous leaf extracts shows the presence of the potent antioxidant property. Most of the tannins have antibacterial, antifungal and anticancer properties. Tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. This tannin-protein complex can provide persistent antioxidant activity.[35]

The total flavonols content was estimated to be 25.69 mg/g for methanolic extracts and 27.59 mg/g for aqueous extracts (Table 2). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for a free-radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS.[35]

The total alkaloid of *Cerbera odollam G* leaf extract was estimated to be 5.21 mg/g for methanolic extracts and 13.51 mg/g for aqueous extracts (Table 2). Most of the alkaloids have local anesthetics and stimulant properties. The alkaloids show cytotoxic activity even in low concentration and other biological activity, showing a wide use in the medical application. Most of the alkaloids are toxic to the human and other organism so it is widely used in medical applications. Alkaloids also show antioxidant properties and anticancer properties like boldine.[48]

The total anthocyanin content of leaf extracts of *Cerbera odollam G was* estimated to be 0.301 mg/g dry weight for methanolic extracts and 0.286 mg/g for aqueous extracts (Table 2). As most of the anthocyanin are coloured pigments found in flower and fruits, the content of anthocyanin was found to be very low in our extracts. Anthocyanin studies have shown monoamine oxidase inhibitor activity connected to the functions in neurogenerative diseases, depression, and anxiety along with neuroprotective and anti-inflammatory activities. It has also been suggested that they show radical scavenging properties and anticancer properties.[49,50]

The total cardiac glycoside content for dry weight of leaf extracts of *Cerbera odollam G was* estimated to be 0.162 mg/g for methanolic extracts and 0.137 mg/g for aqueous extracts. (Table 2). The amount of cardiac glycosides detected in leaf extracts is minor as against larger quantities that have been reported in the fruits of *Cerbera odollam*.[1] This makes the fruits considerably toxic since glycosides act as sodium potassium ATPase inhibitor leading to cell death. Cardiac glycosides are used for the treatment of congestive heart failure and cardiac arrhythmia. They also have anticancer properties.[51]

Inhibition concentration is the amount of free radicals scavenged in the determination of antioxidant activity. Phytochemicals act as antioxidants by scavenging the free radicals.

DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non-radical form DPPH-H.[35] The inhibition concentration of DPPH radical scavenging assay for 5μg of leaf extract of *Cerbera odollam G* was found to be 80.03% for methanolic extracts and 88.38% for aqueous extract. (Table 3), as compared to inhibition concentration for 5μg of ascorbic acid that was found to be 88.89%. Aqueous extract shows higher inhibition concentration and scavenged maximum amount of radicals as compared to methanolic leaf extract. The DPPH scavenging activity can be correlated to the presence of flavonoids, showing higher radical scavenging by aqueous extracts.[52,53,54]

H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage. Antioxidants scavenge hydroxyl radicals.[30] The inhibition concentration of H_2O_2 radical scavenging assay for 20μg of leaf extract of *Cerbera odollam G* (Table 3) was found to be 49.78% for methanolic extracts and 70.82% for aqueous extract. As compared to inhibition concentration for 20μg of ascorbic acid that was found to be 86.47 %. Aqueous extract shows higher inhibition concentration and scavenged maximum amount of radicals as compared to methanolic extract. Hydrogen peroxide radical scavenging activity is correlated to the presence of total phenol content. Hence the radical scavenging activity is higher in aqueous leaf extracts.[52,53,54]

Although superoxide anion is a weak oxidant, it gives rise to a generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. The SO radical scavenging activity in 20μg of leaf extracts of *Cerbera odollam G* was estimated to be 94.57 % in methanolic extract and 91.37 % in aqueous extract indicating a slight difference between the two extracts (Table 3), as compared to 20μg ascorbic acid which was found to be 51.61%. SO scavenging activity is correlated to total flavonoids which are greater in aqueous extract but the radical scavenging activity is greater in methanol extract with slight differences in the percentage of inhibition concentration as compared to aqueous extracts. This indicates the presence of some other compound leading to higher radical scavenging activity in methanol extracts as

Table 1: Result of phytochemical screening of leaf extract of *Cerbera odollam Gaertn.*

Phytochemicals test	Methanol extract	Aqueous extract	Phytochemicals test	Methanol extract	Aqueous extract
Test for alkaloid:	+	+	Test for sterols:	-	-
Test for flavonoids :	+	+	Test for terpenoids:	+	+
Test for saponins:	-	-	Test for quinones:	+	+
Test for tannins:	+	+	Test for oxalate:	+	+
Test for carbohydrates:	+	+	Test for proteins:	+	+
Test for phenols:	+	+	Test for phlobatannins:	-	-
Test for cardiac glycosides:	+	+	Test for betacyanins :	+	+
Test for amino acid:	+	+	Test for fatty acids:	+	+

Where, "+" is present and "-" is absent.

Table 2: Phytochemical constituents in leaf extract of *Cerbera odollam Gaertn.*

Tests	Standard equivalent in methanolic extract.	Standard equivalent in aqueous extract.
Total phenol content	72.167 ± 0.142 mg/g	78.463 ± 0.0149 mg/g
Total tannin content	44.691 ± 0.058 mg/g	90.987 ± 0.115 mg/g
Total flavonoid content	25.698 ± 0.025 mg/g	27.591 ± 0.033 mg/g
Total alkaloid content	5.205 ± 1.39 mg/g	13.511 ± 1.43 mg/g
Total anthocyanin content	0.3013 ± 0.017 mg/g	0.2861 ± 0.104 mg/g
Total cardiac glycoside content	0.1618 ± 0.0036 mg/g	0.1372 ± 0.0065 mg/g

Table 3: Inhibitory concentration values for different test performed, in leaf extract of *Cerbera odallam Gaertn.*

Radical scavenging assay	Ascorbic acid (IC %)	Standard equivalent in methanolic extract. (IC %)	Standard equivalent in aqueous extract. (IC %)
DPPH	88.849 % at 5µg/ml	80.029% at 5µg/ml	88.381% at 5µg/ml
H_2O_2	86.470 % at 20µg/ml	49.781 % at 20µg/ml	70.818 % at 20µg/ml
Super oxide	51.610 % at 20µg/ml	94.567 % at 20µg/ml	91.368 % at 20µg/ml
Nitric oxide	92.718 % at 20µg/ml	95.828 % at 20µg/ml	95.448 % at 20µg/ml
Reducing power assay	N.A	0.0486 ± 0.00252 mg/g	0.049 ± 0.00153 mg/g

Where, "IC %" is Inhibition concentration %

compared to higher total flavonoids content in aqueous extracts of *C. odollam*.[55,35]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions. Antioxidants act by scavenging the NO radical.[35] The NO radical scavenging activity (Table 3) in 20*µ*g of leaf extracts of *Cerbera odollam G* was estimated to be 95.83 % in methanolic extract and 95.45 % in aqueous extract indicating a slight difference between the two extracts, as compared to 20*µ*g ascorbic acid found to be 92.72%. NO radical scavenging activity is correlated to the presence of phenols and flavonoid compounds, which is greater in aqueous extract but the NO radical scavenging activity is greater in methanol extract with slight differences in the percentage of inhibition concentration as compared to aqueous extracts. This indicates the presence of some other compound leading to higher radical scavenging activity in methanol extracts as compared to higher phenols and flavonoids content in aqueous extracts.[56]

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants.[35] The reducing power in 1gm of extracts of *Cerbera odollam G* was estimated to be 0.049 mg/g for methanolic leaf extracts and 0.049 mg/g for aqueous leaf extracts, (Table 3) ascorbic acid equivalent. The reducing power is mainly correlated to the presence of reductones F which are strong reducing agents like ascorbic acid, reductive acid, tartronaldehyde, oxalic acid and formic acid.[57]

Antibacterial and antifungal screening of *Cerbera odollam G*

Zone of inhibition shown by methanol leaf extract of *Cerbera odollam G indicates* the presence of low antibacterial activity as compared to aqueous leaf extract, which shows no zone of inhibition. These indicate that plant extracts had no antibacterial property. (Table 4)

Zone of inhibition shown by methanol leaf extract of *Cerbera odollam G indicates* the presence of good antifungal activity better than standard antifungal drug fluconazole as compared to aqueous leaf extract which shows low to the medium zone of inhibition. These indicate that leaf extracts hold a great potential to use as antifungal agents against the fungus like *Saccharomyces cerevisiae* and *Candida albicans*. (Table 5 and Figure 1 and 2).

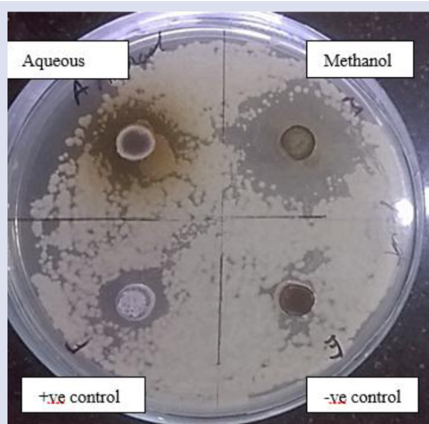

Figure 1: zone of inhibition for the different extracts, against *Saccharomyces cerevisiae*.

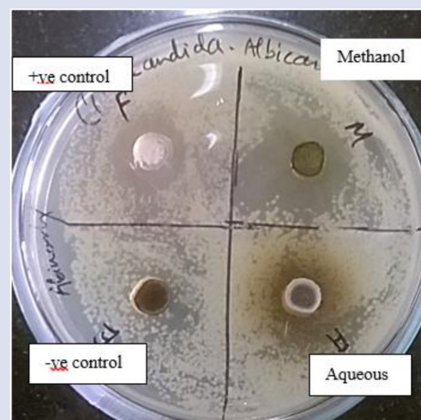

Figure 2: zone of inhibition for the different extracts, against *Candida albicans*.

Table 4: Results of antibacterial assay by agar well diffusion method of *Cerbera odollam G*.

Organisms	Zone of Inhibition (mm)		
	Tetracycline (positive control)	Leaf Methanol extract	Leaf Aqueous extract
Corynebacterium diphtheria	30	Nil	Nil
Bacillus subtilis	22	2	Nil
Staphylococcus aureus	25	2	Nil
Salmonella typhi	27	3	Nil
Klebsiella pneumonia	23	Nil	Nil
Escherichia coli	25	2	Nil

Table 5: Results of antifungal assay by agar well diffusion method for extracts of *Cerbera odollam G*.

Organisms	Zone of Inhibition (mm)		
	Fluconazole (positive control)	Leaf Methanol extract	Leaf Aqueous extract
Saccharomyces cerevisiae	15mm	26mm	16mm
Candida albicans	28mm	25mm	9mm

CONCLUSION

In the current investigation, the extracts from *Cerbera odollam G were* found to be rich in secondary metabolites and possess a significant amount of phytochemicals, antioxidant activity, and antifungal activity. The aqueous extracts showed the higher content of phytochemical constituents like phenols, tannins, flavonols and alkaloids, and higher antioxidant activity for DPPH radical scavenging assay, H_2O_2 radical scavenging assay and reducing power assay than methanol leaf extract. The methanol extract showed the higher content of anthocyanin, cardiac glycoside, and higher antioxidant activity for superoxide radical scavenging assay and nitric oxide radical scavenging assay than aqueous leaf extracts. The antioxidant mechanisms of the leaf extracts may be attributed to their free radical-scavenging ability. In addition, phenolic compounds and other phytochemicals appear to be responsible for the antioxidant activity of the extracts. The anti-microbial activity was found to be higher in the methanolic extract as compared to aqueous leaf extract with a low zone of inhibition against bacteria and moderate to the high zone of inhibition against fungus. On the basis of the results obtained, the leaf is rich sources of natural antioxidants and could be developed into drug against diseases such as inflammation, diabetes, cardiac arrest, and hypertension for a variety of beneficial chemo-preventive effects. Isolation and purification of phytochemicals and antifungal constituents of the plant and evaluation of minimum inhibitory concentration (MIC) will provide with an alternate to an existing product and its efficiency.

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ABBREVIATIONS USED

v/v: volume by volume; **NBT:** Nitro Blue Tetrazolium; **NADH:** Nicotinamide Adenine Dinucleotide; **PMS:** Phenazine Metho Sulphate; **ROS:** Reactive Oxygen Species.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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SUMMARY

- Most of the phytochemicals were found to be higher in aqueous extract. Cardiac glycoside were found to be higher in methanol extract.
- Antioxidant assay involving DPPH, H_2O_2 & reducing power, radical scavenging assay where higher for aqueous extract. SO & NO radical scavenging assay were found to be higher in methanol extract. Exhibiting potent antioxidant properties.
- Anti-microbial assay where found to be higher for methanol extract, exhibiting higher antifungal and lower antibacterial properties.

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