



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

International Journal of Current Research

Vol. 10, Issue, 12, pp.76234-76237, December, 2018

DOI: <https://doi.org/10.24941/ijcr.33484.12.2018>

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES IN TUBER EXTRACT OF *CORALLOCARPUS EPIGAEUS* (ROTTL. AND WILLD.) HOOK. F.

¹Patil, U. S. and ²Kutemate, O. G.

¹Associate Professor and Head, Department of Botany, Bharatiya Mahavidyalaya, Amravati

²Research student, Department of Botany, Bharatiya Mahavidyalaya, Amravati

ARTICLE INFO

Article History:

Received 26th September, 2018

Received in revised form

14th October, 2018

Accepted 29th November, 2018

Published online 31st December, 2018

Key Words:

Corallocarpus Epigaeus (Rottl. & Willd.)
Hook. f. tuber, Preliminary Phytochemical
Screening, Antimicrobial Activity.

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Citation: Patil, U. S. and Kutemate, O. G. 2018. "Preliminary phytochemical screening and antimicrobial studies in tuber extract of *Corallocarpus epigaeus* (Rottl. and Willd.) Hook. f.", *International Journal of Current Research*, 10, (12), 76234-76237.

ABSTRACT

The present study attempt to evaluate the preliminary phytochemical screening and antimicrobial studies in tuber extract of *Corallocarpus epigaeus* (Rottl. and Willd.) Hook. f. Qualitative phytochemical analysis of samples in different solvent extract confirms the presence of various phytochemicals like Carbohydrates, Saponins, Tannins, Flavonoid, Alkaloids, Glycosides, Proteins, Phytosterol, Steroids, Phenols and Terpenoids. The solvent Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate and aqueous extract showed good results. Disk diffusion method was used to study the antibacterial activity against *Salmonella tify*, *Escherichia coli*, *Streptococcus pyogenes*, antifungal activity against *Candida albicans*, and antiprotozoal activity against *Entamoeba histolytica*. It has been observed that the samples in different solvent tuber extract showed antibacterial, antifungal, and antiprotozoal activities by the formation of inhibitory zone. It may be attributed due to the presence of phytochemicals and may be used as antimicrobial agents.

INTRODUCTION

Plant medicines are nature's gift, which has bestowed its blessings to cure many diseases. Plants contain many chemical compounds of therapeutic use which are known as phytochemicals. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans (Hasler and Blumberg, 1999). Secondary plant metabolites are the basis of treatment of many microbial diseases in humans. Hence in recent years plant medicines are being used in treatment of various microbial ailments. *Corallocarpus epigeous* (Rottl and Willd.) Hook. f. belongs to the family Cucurbitaceae. The stems are glabrous, much branched, somewhat zigzag or angular at nodes. Leaves 3-5 lobed or angular, cordate, acute or rounded and obtuse sub entire sinuous to minutely, irregularly dentate, short hispid-to long hirsute-scabrid on both surfaces; petiole 1-1.5cm long, hispid to sub glabrous. Male flowers: peduncle 2-4 cm long, glabrous. Female flowers: peduncles 0.2-0.6 cm long, stout. Fruit 1.7-3 x 0.8 - 13cm including beak 0.6- 0.8mm long, ovoid, circumcissile with line of dehiscence 2-3mm above base. Seeds few, 4-7 longer than broad. The herb is distributed both in Central Africa and Asia, growing in rich but well drained soil with some water Kamble and Rao (2018).

*Corresponding author: Patil, U.S.,

Associate Professor and Head, Department of Botany, Bharatiya Mahavidyalaya, Amravati.

It is a traditionally used in siddha and ayurveda as an antivenom for snakebite but it is not much exploited much in modern medicine. The tubers are used in the treatment of chronic rheumatism, snakebite, asthma and syphilitic disorder (Saranya *et al.* 2015). The rhizome is especially useful in Syphilitic cases, old venereal complaints, chronic dysentery and snake bite (Kirtikar and Basu, 1996). Anti diabetic activity in *Corallocarpus epigaeus* (Rottl. and Willd.) Hook. f. tuber was checked by Kattamanchi Gnananath *et al.*(2013).The antioxidant and anti-inflammatory activities of *Corallocarpus epigaeus* (Rottl. and Willd.) Hook. f. tubers were evaluated, where ethanol extract inhibited significant anti-inflammatory activity (M.Jayaseelan *et al.* 2014). In vivo screening of *corallocarpus epigaeus* (Rottl and Willd.) Hook. f. tuber for its analgesic, anti-pyretic and anti-inflammatory activities was done and analgesic activity, anti-inflammatory property similar to steroidal and non-steroidal agents as well as antipyretic effect were determined by Naik *et al.* (2012). The above work promotes for phytochemical screening of tuber extract in *corallocarpus epigaeus* (Rottl. and Willd.) Hook. f. for the identification of potential phytochemicals responsible for medicinal properties and as antimicrobial agents. Present work was carried out to screen the preliminary phytochemical compound and to evaluate its antimicrobial activities. Three bacterial species, one fungal strains and one protozoal stain were used for antimicrobial studies.

MATERIALS AND METHODS

Plant material: The whole plant of *coralocarpus epigaeus* (Rottl. and Willd.) Hook. f. was collected in fresh condition from southern part of Melghat, Buldana District, Maharashtra, India. The plant was identified authenticated by well known taxonomist Dr. S. M. Bhuskute Principal, Bhavbhuti Mahavidyalaya, Amgaon, district Gondia, Maharashtra. Voucher specimen has been deposited in the department of Botany, Bhartiya Mahavidyalaya, Amravati, Maharashtra. The tubers were washed under running water and dried under shade, then ground into a fine powder by using blender and stored in plastic bottle at room temperature.

Preparation of extracts: The extraction of soluble compounds from tuber of *coralocarpus epigaeus* (Rottl. and Willd.) Hook. f. was performed by using the soxhlet's extractor method with various solvents like Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate and Distilled water. 25 gms of dried powder were taken in a cone made from Whatman filter paper No.1 and placed into soxhlet's apparatus. 100 ml of above solvents were taken successively, in the round bottom flask attached to the soxhlet apparatus. A condenser was attached to this setup. Then the whole setup was placed on a heating mantle. The temperature was set in the range where the solvents gets vaporized and rises up to the condenser where it condenses back into liquid. This liquid falls into the plant sample in the cone and extracts certain compounds and falls back into the round bottom flask. This process was continued till all the compounds get's extracted from the sample. The extracts obtained from the above process was evaporated and stored in cap glass vials.

Phytochemical analysis: Preliminary phytochemical screening of samples was carried out with the following methods described by Harborne, J.B. (1973).

Test for Detection of carbohydrates: Molisch's Test: Small Quantity of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extract were taken separately in 10 ml of distil water and two drops of Ethanolic naphthol (20%) and 2ml of concentrated Sulphuric acid were added, formation of reddish violet ring at the junction indicates presence of carbohydrates.

Test for Detection of Saponins: Foam Test: 2 ml of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extract were taken and added equal amount of distil water and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins (Kumar *et al.*, 2009).

Test for Detection of Tannins: Ferric Chloride Test: Small Quantity of Petroleum ether, Benzene, Acetone Chloroform, Ethyl acetate, Distil water extract were taken separately in water, 2-3 drops of 5% ferric chloride was added. Formation of black or green colour indicates the presence of tannins.

Test for Detection of Flavonoids: Sulphuric Acid Test: A fraction of extract was treated with concentrated sulphuric acid and observed for formation of orange colour.

Test for Detection of Alkaloids: Mayer's Test: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added then few drops of Mayer's reagent were added.

Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Detection of Glycosides: Sulphuric Acid Test: To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added then few drops of concentrated sulphuric acid were added. Presence of greenish blue colour indicates the presence of glycosides.

Test for Detection of Proteins and Amino acids: Ninhydrin Test: To 2ml of plant extract, few drops of 0.2% Ninhydrin was added and heated for five minutes. Formation of blue colour indicates presence of proteins.

Test for Detection of Steroids and phytosterols: Sulphuric Acid Test: To 1 ml of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.

Test for Detection of Phenols: Ferric Chloride Test: To 1 ml of plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicates presence of phenols.

Test for Detection of Terpenoids: Salkowski test : 2ml of plant extract was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. Reddish brown colouration of the interface is formed indicating the presence of terpenoids.

Antimicrobial Activity: The role of medicinal plants is very important in treatment of various diseases in human being. Medicinal plants are the first home remedy that a person relies on for hepatic as well as gastrointestinal troubles. Ginger and garlic are home remedies against gaseous trouble, indigestion etc. Though antifungal and antibacterial drugs control infections but the pathogen become resistant to these allopathic drugs, second chance is the infection gets cured causing other complications and side effects. To prevent patient from drug resistant species and undesirable side effects plant medicine prove to be the best solution. Higher plants have shown to be potential source of new antimicrobial agent (Mitscer, 1987). Especially hepatic and gastrointestinal disorders are challenge before modern medicine, hence scientist are showing great interest for the discovery of new effective plant based drugs for treatment of these disorders.

Collection of Bacterial and Parasitic Isolates: Bacterial isolates of *Escherichia coli*, *Salmonella typhi*, *Streptococcus pyogenes*, *Candida fungus* and parasitic isolates of *Entamoeba histolytica*, were obtained from Samruddhi Microbiology Diagnostic Laboratory, Amravati which is run by Dr. S. R. Gulhane (Microbiologist). The isolates were authenticated by biochemical tests as described by Cheesebrough (1985) preserved on potato dextrose agar and nutrient agar respectively and stored at 4°C until ready to use.

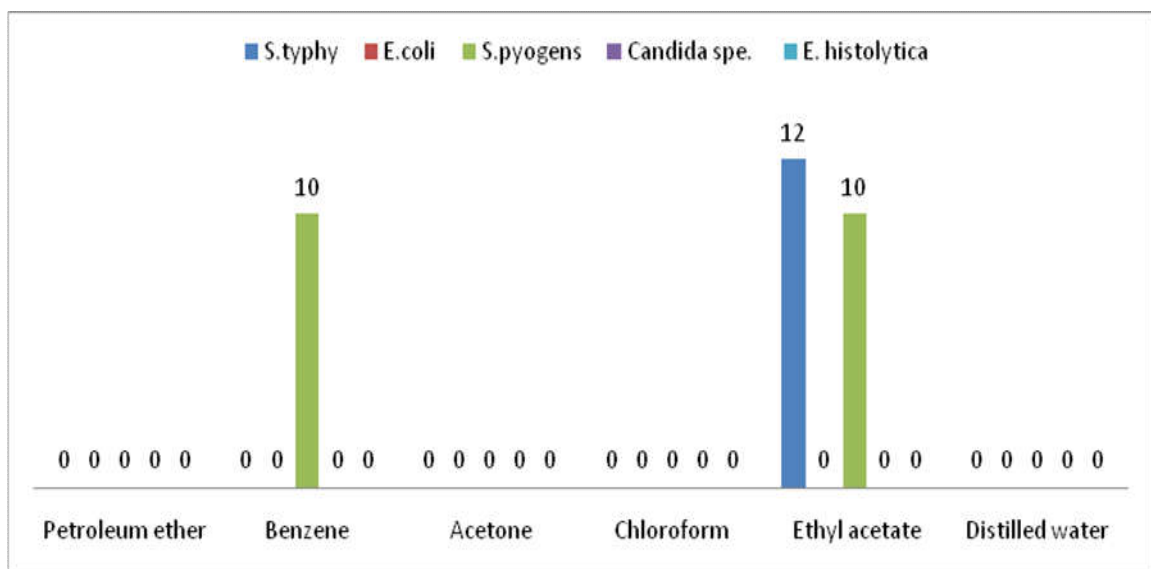
Disc Diffusion Method: The bacterial isolates of *Escherichia coli*, *Salmonella tiphy*, *Streptococci pyogenes*, *Candida fungus* and parasitic isolates of *Entamoeba histolytica*, were subculture overnight at 37°C on potato dextrose agar and nutrient agar plates respectively. Six plate per organism.

Table 1. Preliminary phytochemical screening of *Corallocarpus epigaeus*(Rottl. & Willd.) Hook. f.

S. No.	Secondary metabolite	Petroleum ether	Benzene	Acetone	Chloroform	Ethyl acetate	Distilled water
1	Carbohydrates	---	+	++	++	+++	+++
2	Saponins	+	+++	---	++	---	---
3	Tannins	---	---	---	---	---	+
4	Flavonoids	---	---	++	+	++	+++
5	Alkaloids (Mayer's test)	---	+++	+	+	---	+++
6	Glycosides	+	---	---	---	---	---
7	Proteins	---	---	---	---	---	---
8	Phytosterol & Steroids	---	++	+	+	---	+++
9	Phenols	---	---	---	---	---	---
10	Terpenoids	+++	+	++	+++	+++	++

Table 2. Antimicrobial activity in tuber extract of *Corallocarpus epigaeus* (Rottl. & Willd.) Hook. f.

Microorganism	Petroleum ether	Benzene	Acetone	Chloroform	Ethyl acetate	Distilled water
<i>S.typhy</i>	00	00	00	00	12mm	00
<i>E.coli</i>	00	00	00	00	00	00
<i>S.pyogens</i>	00	10mm	00	00	10mm	00
<i>Candida spe.</i>	00	00	00	00	00	00
<i>E. histolytica</i>	00	00	00	00	00	00

**Table 3. Analysis of antimicrobial sensitivity of *Corallocarpus epigaeus*(Rottl. & Willd.) Hook. f.(tuber)****Fig. 1. *Corallocarpus epigaeus* (Rottl. & Willd.) Hook. f.****Fig. 2. Tuber****Fig. 3. Antimicrobial plates**

The suspension of each bacterial and parasitic isolates were prepared as described by John *et al.* (1999) in isotonic sodium chloride solution. Solidified petridishes, for each microorganism for six solvents on Muller- Hinton agar were flooded with the appropriate suspension of bacterial isolates respectively. Sterile 6 mm diameter absorbent filter papers disc (punched out from No.1 Whatman paper) were impregnated with respective solvents of plant extracts namely Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distilled water extracts and placed on inoculated lawn. Six extracts from each plant parts in ten plants were tested for antimicrobial sensitivity. All the plates were kept for incubation period i.e. 24 hrs. for bacteria and parasites respectively at room temperature. Results were noted down in terms of sensitivity zone around the disc which is measured in millimeter (mm) and results were sequentially recorded in the tabular form.

RESULTS AND DISCUSSION

Table 1 represents various photochemicals present in different extracts. The *Corallocaepus epigaeus* (Rottl. and Willd.) Hook. f. tuber extract in petroleum ether shows presence of saponins, glycosides and terpenoids. While the extract in benzene shows the presence of Carbohydrates, saponins, alkaloids, terpenoids and phytosterols. Acetone extract depicts the presence of carbohydrate, flavonoids, alkaloids, phytosterols and terpenoids. The presence of carbohydrates, saponins, flavonoids, alkaloids, phytosterols and terpenoids was determined in chloroform extract. The ethyl acetate shows the presence of carbohydrates, flavonoids and terpenoids. Significant amount of carbohydrates, tannins, flavonoids, alkaloids, phytosterols and terpenoids were present in aqueous extract of *Corallocaepus epigaeus* (Rottl. and Willd.) Hook. f. tubers. The benzene extract showed inhibitory activity against *S. pyogenes* with a zone of inhibition of 10mm. The extract in ethyl acetate showed positive result against *S. typhi* and *S. pyogenes* with a maximum zone of inhibition of 12mm and 10mm respectively. Terpenoids extracted in all solvents in moderate as well as abundant amount. Terpenoids have been found to inhibit growth of cancerous cells and also decrease microorganism concentration (Gupta *et al.* 2011). The extract in petroleum ether, acetone, chloroform and distilled water did not show any antimicrobial activity.

Conclusion

The result of present study clearly indicates that, the preliminary phytochemical analysis in tuber extracts of *Corallocaepus epigaeus* (Rottl. and Willd.) Hook. f. shows presence of rich amount of metabolites like Carbohydrates, Saponins, Tannins, Flavonoid, Alkaloids, Glycosides, Phytosterol, Steroids, Phenols, and Terpenoids. Tuber extract of *Corallocaepus epigaeus* in benzene and ethyl acetate showed good antibacterial activity and it may be attributed due to the presence of phytochemicals and may be used as antimicrobial agents.

Acknowledgement: Authors feel deeply obliged to Dr. S. M. Bhuskute Principal, Bhavbhuti Mahavidyalaya, Amgaon, district Gondia, M.S. for his kind help in identification of plant specimen.

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