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In vitro Anti-Oxidant Studies by Using Different Methods and Evaluation of Anti-Microbial Potential of *Coptis teeta*

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Abstract: There are plenty of antioxidant substances present in plants parts such as fruits, vegetables, medicinal herbs and the free radical scavenging molecules present in them are in the form of Phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), Nitrogen compounds (alkaloids, amines), Vitamins, Terpenoids (carotenoids) and some other endogenous metabolites. Therapeutically properties of medical plants are very useful in healing various diseases and the advantages of these medicinal plants are natural. In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years. Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, viral and microbial infections. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The present investigation was carried out to evaluate the antioxidant activity and antimicrobial activities of ethanolic root extract from *Coptis teeta.*The preliminary phytochemical analysis showed the presence of an alkaloids, flavonoids, glycosides in the dried ethanol root extract of *Coptis teeta*. The antioxidant activity has been estimated using the *in vitro* methods like DPPH and Nitric Oxide free radical scavenging activity. It showed that the ethanolic root extract from C*optis teeta* showed significant free radical scavenging activity.

Key words: C*optis teeta* · Antioxidant Activity · Medicinal Plants · Antimicrobial Activity

INTRODUCTION

There is a strong need to adopt modern analytical methods for standardization and quality evaluation of herbal drugs to ensure their therapeutic efficacy [1]. Plants are used medicinally in different countries are source of many potent and powerful drugs [2]. Natural products are extremely important as sources of medicinal agents, model for the design of synthetic and semi synthetic novel substances for treating human diseases [3]. There are plenty of antioxidant substances and free radical scavenging molecules present in the plants are in the form of phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (carotenoids) and some other endogenous metabolites [4]. The medicinal herbs exhibited stronger antioxidant activity and contained significantly higher levels of phenolics than common vegetables and fruits [5, 6]. The potency of radical scavenging effect of *M. officinalis* extract has about four times greater than synthetic antioxidant butylatedhydroxy toluene [7]. In the emulsion system, only apigenin has pro-oxidative activity while other flavones and flavonols plant extracts inhibited oxidation of β-carotene [8]. Three methods widely employed in the evaluation of antioxidant activity namely 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, static headspace gas chromatography (HS-GC) and β-carotene bleaching test (BCBT) [9]. The root contains several compounds that are safe and effective in inhibiting bacterial disease such as dysentery [10]. *Coptis teeta* is a rare species of flowering plant in the buttercup family and important in Chinese herbology known as Yunnan gold thread a rhizome used as an antimicrobial and anti-inflammatory agent [11]. *Coptis teeta* wall is widely used for ocular ailments in the Unani system of medicine as a drug of choice and has proved beneficially safe [12]. The key alkaloid contents in leaf

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and fibrous root were unconventional medicinal parts. The biomass and alkaloids in individual plant presents significantly positive correlation [13]. *Coptis teeta* has antifungal and antibacterial properties can cure cold, fevers, tropical diarrhea and inflammation of eyes, whooping cough, diphtheria and typhoid fevers while rhizomes contain alkaloids of Berberine group, which is a primary constituent and can decrease ventricular fibrillation, releases arachidonic acid from cell membranes and inhibits platelet aggregation [14]. The antioxidant activities of four medicinal plants traditionally used in the treatment of malaria in southwestern Nigeria were determined [15]. Amongst these are plants used for the management of neurodegenerative diseases such as Parkinson's, Alzheimer's, loss of memory, degeneration of nerves and other neuronal disorders by the Ayurvedic practitioners [16].

MATERIALS AND METHODS

Ethanolic Extract of Coptis Teeta: The root of *Coptis teeta* were obtained from Uwin Life Science Malappuram. The obtained root were cleaned from dust and other materials then dried at the room temperature. The dried roots were pulverized in an electric grinder. The powdered material was soaked in 90% ethanol for four days by stirring the mixture twice daily. After fourth day, the mixture was filtered then marc was pressed and this process was repeated three times. All the alcoholic fractions were combined and the ethanol was subjected for evaporation. The syrupy consistency material obtained was heated on the water bath until dry extract was obtained. The obtained ethanolic root extract of *Coptis teeta* were labelled and stored in the desiccators for further usage.

Preliminary Qualitative Phytochemical Analysis: The ethanolic root extract of *Coptis teeta* were subjected to qualitative examination for different phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Proteins, Saponins, Terpenoids and Steroids by using standard methods [17, 18].

Test for Carbohydrates: Molisch's test: Small quantity of 300mg alcoholic extract and dried leaf extract powder of Pimentadioica were separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test. The formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's Test: Small portion of extract is dissolved in water and treated with Fehling's solution [brown color indicated the presence of carbohydrate.

Phenols Test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. The blue coloration of the spot indicated the presence of phenols.

Test for Flavonoids

Shinoda Test: A piece of magnesium ribbon and 1ml of concentrated HCl was added to 3ml of extract. A pink or red coloration of the solution indicated the presence of flavonoids in the drug.

Lead Acetate Test: 1ml of lead acetate solution was added to 5ml of extract. The flocculent white precipitate indicated the presence of flavonoids.

Test for Tannins: Braemer's test: 10% alcoholic ferric chloride solution was added to 3ml of the extract. A Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for Alkaloids:

Draggendorf's Test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent. Orange coloration of the spot indicated the presence of alkaloids.

Wagner's Test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Tests for Glycosides:

Legal's Test: 0.1g of extract was dissolved in 2ml of pyridine containing 2ml of sodium nitroprusside solution and were made alkaline with Sodium hydroxide solution. A pink to red color solution indicates the presence of glycosides.

Test for Saponins:

Foam Test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes. A1cm layer of foam formation indicates the presence of Saponins.

Test for Anthraquinones:

Borntrager's Test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the

filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Steroid/terpenoid:

Libermann-burchardt Test: 1ml of extract, 1ml of chloroform, 3ml of acetic anhydride and 2 drops of concentrated Sulphuric acid are added to 1ml of extract. A dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for Amino Acids:

Ninhydrin Test: Small quantity of the extract was dissolved in few ml of water and 1ml of ninhydrin reagent was added. A blue color indicated the presence of amino acids.

Test for Fixed Oils and Fats: Small quantity of the petroleum ether extract was pressed between two filter paper. An oil stains on the paper indicated the presence of fixed oils.

***In Vitro* Antioxidant Study:** *In vitro* methods were performed [19] for the estimation of antioxidant activity of *Coptis teeta* root, by DPPH and Nitric Oxide radical scavenging assay. Free radical scavenging activity was measured by a decrease in absorbance at 517nm of coloured DPPH in methanol solution. A stock solution of DPPH (1.3 mg/ml in methanol) was prepared from that 75µl of it in 3ml methanol gave an initial absorbance of 0.9nm. The decrease in the absorbance in the presence of sample extract and standard at different concentrations was noted after 30 minutes. The IC_{50} was calculated from % inhibition. A blank reading was taken using methanol instead of sample extract. Absorbance at 517 nm is determined after 30 minutes by using UV-visible Spectrometer (Systronic double beam- UV-2201) and IC50 (Inhibitory concentration to scavenge 50% free radicals) was also determined. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{Percentage Inhibition} = {}^{C-T} \times \frac{100}{A}$$

Where,

C = Absorbance of DPPH alone, T = Absorbance of DPPH along with different concentrations of extracts, IC50 was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition.

Nitric Oxide Radical Scavenging Assay: Five mg of extract was dissolved and mad up to 10ml with methanol. The sample was completely soluble. 50µl of 10mM Sodium nitroprusside and 50µl test solution of various concentrations are illuminated using fluorescence light at room temperature for 150 minutes. Following incubation, 125µl of Griess reagent was added and incubated for 30 minutes at room temperature. The absorbance was measured at 546nm.

Evaluation of Anti-microbial Activity of the Plant Using Disc Diffusion Method: The methanol extracts of *Coptis teeta* were evaporated using Lyophilizer. Different concentrations were prepared by using alcohol. An overnight bacterial suspension (100µl) adjusted to contain 1x106 CFU/ml of bacteria, spread with the help of sterile cotton swab on Muller Hinton agar medium. Different concentrations were placed in the wells and were incubated at 27°C for 24 hours [20]. After incubation the inhibition zones were measured in diameter (mm). An antibiotic disc containing 1µg of Ciproflaxin was used as positive control and DMSO used as negative control. The minimal inhibitory concentration was calculated.

RESULTS

Plant Description:

Botanical Name: Ranunculaceae/Yunnan gold thread/Coptis teeta, Family: Ranunculaceae, Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Ranunculales, Genus: Coptis, Species: *C. teeta*

Preliminary Phytochemical Screening: After performing the preliminary phytochemical screening it was observed that *Coptis teeta* showed the negative interpretation for the carbohydrates for molishch's test and Fehling's test, phenols for phosphomolybdic acid, tannins for Braemer's test, saponins for foam's test, steroid for Libermann-Burchardt test, amino acids for ninhydrin test and detection of oils and fats for spot test while positive interpretation for the flavonoids for Shinoda`s test and lead acetate test, alkaloids for Wagner,s test and Draggendrof's test, glycosides in trace amount for Legal's test

Table 1: DPPH free radical scavenging activity

Concentration (µg/ ml)	Optical Density	Percentage Inhibition (%)
00	1.34	00
32	1.23	8.2
64	0.86	35.8
96	0.68	49.2
128	0.45	68.46

Table 2: Nitric Oxide free radical scavenging activity

Concentration (µg/ ml)	Optical Density	Percentage Inhibition (%)
00	1.16	00
32	0.85	26.7
64	0.65	43.9
96	0.54	53.4

***In Vitro* Antioxidant Study:** The antioxidant activity of *Coptis teeta* by DPPH are present in Table 1 and nitric acid scavenging activity are presented in Table 2.

The Anti-microbial Study of *Coptis Teeta:* The antimicrobial activity of the *Coptis teeta* by well method are represented in Table 3.

DISCUSSION

***Coptis Teeta* Are Called by Common Name:** Yun lian in Arabic, Mamiranchini in Assamese, Misimitita in Hindi, Mishmitita in Malayalam, Pitarohini in Sanskrit, Pitarohini in Tamil and Mameeran in Urdu. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent and can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell [21]. *Coptis teeta's* major active compounds such as berberine, jatrorrhizine and palmatine are determined in seven different genuine producing areas by using HPLC method has many advantages [22]. Being rapid, simple and independent of sample polarity, the DPPH method is very convenient for the quick screening of many samples for radical scavenging activity [23]. The most frequently used plant parts were leaves 33%, roots 31% and bark as well as whole plant are 12% [24]. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation react ions [25]. They do this by being oxidized themselves, so antioxidants are often reducing agents. An antioxidant is a molecule that inhibits the oxidation of other, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A and vitamin E as well as enzymes such as catalase, superoxide-- dismutase and various peroxidases [26]. The preliminary phytochemical analysis showed the presence alkaloids, flavonoids, glycosides in the dried root ethanol extract *Coptis teeta* [16]. The free radical scavenging capability of the plant was determined using two *in vitro* methods [27]. The study showed that the extract possess significant free radical scavenging activity as it showed a valuable IC_{50} against the *in vitro* results [7]. Extracts obtained by organic solvents from the root of *Coptis teeta* in Myanmar (Burma), were tested for growth inhibitory activity against *Giardia lamblia*, *Trichomonas vaginalis* and *Entamoeba histolytica* in axenic culture [28]. Comparing the inhibitory effects of the methanol extract with berberine sulfate, it was observed that the crude extract was more effective than the salt [29]. At the end of the study we found that the plant *Coptis teeta* is a highly potential medicinal plant having both anti-microbial and anti-oxidant activity [30]. The *in-vitro* activity in-terms of antioxidant assay were confirmed using both DPPH method and Nitric oxide method [19]. Being the plant is a potential one and also using in Ayurvedic systems of medicine the current work supports the scientific validation of the usage of the plant [3]. The invitro antioxidant study have been performed based on the DPPH assay showed an IC_{50} value of 99 mg/ml while *in vitro* antioxidant study have been performed based on the Nitric assay showed an IC_{50} value of 84 mg/ml [9]. Antimicrobial study of the plant using disc diffusion method with different test microorganisms shows high potential of antimicrobial activity.

Table 3: The antimicrobial activity of the *Coptis teeta*

	Concentration of the sample					
Test organisms	10mg	20mg	40mg	60mg	MIC	Ciprofloxacin
Escherichia coli	2mm	2mm	4 mm	6mm	10mg	15mm
Staphylococcus aureus	0mm	2mm	4 mm	10mm	20mg	20mm
Solmonella typhi	0mm	2mm	6mm	10mm	20mg	25mm
Pesudomonas aurenginosa	0mm	0mm	0 mm	8nm	60mg	20mm

CONCLUSION

From the present work we conclude that the ethanolic root extract from *Coptis teeta have* highly potential anti-microbial and anti-oxidant activity. The in-vitro activity in-terms of anti-oxidant assay was confirmed using both DPPH method and Nitric oxide method. Being the plant is a potential one and also using in Ayurvedic systems of medicine the current work supports the scientific validation of the usage of the plant. it will be a boon for the entire pharmaceutical industry. Free radicals are produced in normal or pathological cell metabolism from xenobiotics or through ionizing radiation. Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. As the work revealed that the plant is a highly potential medicinal plant isolation of chemical compounds from the plant will be highly relevant. The present work concluded that the identification and characterization of chemical compounds from the plant will lead to the identification of new active potential compounds and it will be a boon for the entire pharmaceutical industry.

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