Biological Forum – An International Journal **15(2): 1114-1118(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Evaluation of Antioxidant and Antimicrobial Potential of Herbal Phenolics

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(Received: 02 January 2023; Revised: 15 February 2023; Accepted: 17 February 2023; Published: 23 February 2023)
(Published by Research Trend)

ABSTRACT: Antimicrobial resistance is the big challenge across the globe. Infectious and non infectious diseases are the consequences of microbial invasion of human biological system. Worldwide infectious diseases are the second leading cause of death and disability. Antimicrobial resistance is the consequence of inappropriate use of antibiotics in noninfectious disease. Herbal bioactives are the major source of new antimcrobial molecules. Plants are the rich source of secondary metabolites proved therapeutic potential in many diseases. Aim of present study was to evaluate the antioxidant and antimicrobial activity of different extracts of *C. sinensis*. Total Phenolic content of water, hydroalcoholic, alcoholic and ethyl acetate extract was determined by fiolin- Ciocalteu method. Antioxidant activity of all extracts were determined by DPPH method. Antimicrobial activity of all extracts was determined by agar well diffusion method using gram-positive, gram negative bacteria and fungus species. Total Phenolic content was found highest in ethyl acetate fraction. Antioxidant activity of all extract was found highest at a concentration of 50 µg/ml. optimum free radical scavenging effect was found in ethyl acetate fraction. Most significant antimicrobial activity was found against *A. niger*, while antimicrobial activity was not found against *C. albicans, E. coli* and *P. vulgaris*. Free radical scavenging result revealed all the extract possess good antioxidant activity. Amongst all extracts, ethyl acetate extract possess optimum free radical scavenging effect Antimicrobial effect was determined by using seven strain of microorganisms. Minimum inhibitory concentration (MIC) found at 200 µg/ml. The mean diameter of zone of inhibition (ZOI) was found to be dose dependent, maximum ZOI found at 400 µg/ml against *A. niger staphylococcus aureus, Bacillus sustilis, S. epidermidis*. Antioxidant and antimicrobial result reveal that *C. sinensis* could be a good antioxidant and novel safe and effective anti microbial agent could be use in treatment of many infectious disease as a chemotherapeutic agent.

Keywords: Free radical scavenging, antioxidants, bioactive, infectious disease.

INTRODUCTION

The world is facing the challenges of infectious diseases as well as emerging and reemerging diseases. Infectious disease is the most significant cause of morbidity, and mortality accounts for 50 percent of all deaths (Magiorakos *et al.*, 2011). Antimicrobial resistance is a global health threat. The world is facing an alarming increase in antimicrobial resistance (AMR). Plants have been used to treat a variety of diseases since antiquity. For a long time, natural drugs or medicines have been shown to be effective in most infectious diseases. Plant therapies are effective in controlling infections such as topical skin infections, wound infections, minor acute infections, and chronic infections. Plant based medicines play a central role in all traditional systems of medicine. Green tea is a popular drink drunk by people all over the world. *C. sinensis* is well known for its health benefits (Viale *et al*., 2015). There are four types of tea, white tea, prepared from young tea leaves or buds; black tea, prepared from mature fully fermented leaves; green tea, prepared from mature unfermented leaves; and oolong tea, prepared from mature partially fermented leaves (Gupta *et al.,* 2000). Amongst all teas, green tea has potential therapeutic efficacy due to the presence of a rich amount of phenolics and flavonoids (Einother *et al.,* 2013; Konarikova *et al.,* 2015). The main constituents of green tea are (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (Demain, and Fang 2000). EGCG is the most abundant constituent of green tea and constitutes approximately 59% of the total catechins (McNaught, Fernandez *et al.,* 2002; Lin *et al*., 2003; Cabrera *et al*., 2006; Jigisha *et al.,* 2012). Green tea phenolics proved therapeutic efficacy in the management and control of many diseases, such as an antioxidant, anticarcinogenic, antinflamatory, in cardiovascular disorders, neurodegenerative disorders, oral health, Parkinson's disease, Alzheimer's, and as an antimicrobial (Serafini *et al.,* 2011; Subramani and Natesh 2013; Reygaert and Jusufi 2013; Gupta *et al.,* 2014). It is effective in almost all types of cancer (skin, breast, prostate, lung, etc.), reduces atherosclerosis and inhibits the attachment of bacteria to the oral surface,

effective in oral health, it inhibits both gum and tooth caries, thereby preventing tooth decay. It proved to have an antimicrobial effect against a variety of gram-positive positive, gram negative bacteria, fungi, and viruses (Fernandez *et al.,* 2002; Lin *et al.,* 2003; Cabrera *et al*., 2006). A research study showed that oxidative stress results in the development of infectious as well as noninfectious diseases. The antioxidant activity of green tea is attributed to the presence of polyphenols, research evidence showed that EGCG is 20 times more active than vitamin C, 30 times more active than vitamin E (Vinson et al., 1995). Several research studies have been conducted in the past to determine the antimicrobial potential of *C. sinensis* (Das, 1962; Ryu, 1980; Scalbert, 1991; Senthilkumar *et al.,* 2014). An antimicrobial study was carried out on *S. aureus, S. epidermidis, Salmonella typhi, Salmonella typhimurium, Salmonella enteritidis, Shigella flexneri, Shigella dysenteriae,* and *Vibrio* spp. (Toda *et al.,* 1989; Toda *et al*., 1991; Toda *et al.,*1992). A study was carried out on B. pertussis (Horiuchi *et al.,* 1992). Another study was carried out for dental caries (Onishi *et al.,* 1981). One more antibacterial study was conducted on *Streptococcus mutans* (Kawamura *et al.,* 1989). Research studies showed that green tea extracts were effective against *Clostridium* spp., *Erwinia* spp. and *Pseudomonas* spp., including *Vibrio cholera* (Hara *et al*., 1989; Ahn *et al.,* 1990; Ahn *et al.,* 1991; Fukai *et al.,* 1992). Recently, antifungal activity of green tea was conducted on Candida albicans. Research reports showed that green tea extract had good antimicrobial effects on many microorganisms. In this study, the focus was on the antioxidant potential of green tea as well as their antimicrobial effect on different microorganisms. tributed to the presence of polyphenols, research evidence showed that EGCG is 20 times more active than vitamin C, 30 times more active than vitamin E (Vinson *et al.,* 1995). Several research studies have been conducted in the past to determine the antimicrobial potential of *C. sinensis* (Das, 1962; Ryu, 1980; Scalbert, 1991; Senthilkumar *et al.,* 2014). An antimicrobial study was carried out on *S. aureus, S. epidermidis, Salmonella typhi, Salmonella typhimurium*, *Salmonella enteritidis, Shigella flexneri, Shigella dysenteriae, and Vibrio* spp. (Toda *et al*., 1989; Toda *et al*., 1991; Toda *et al*., 1992). A study was carried out on B. pertussis (Horiuchi *et al*., 1992). Another study was carried out for dental caries (Onishi *et al.,* 1981). One more antibacterial study was conducted on Streptococcus mutans (Kawamura *et al*., 1989). Research studies showed that green tea extracts were effective against *Clostridium* spp., *Erwinia* spp. and *Pseudomonas* spp., including *Vibrio cholera* (Hara *et al.,* 1989; Ahn *et al.,* 1990; Ahn *et al.,* 1991; Fukai *et al.,* 1992). Recently, antifungal activity of green tea was conducted on *Candida albicans*. Research reports showed that green tea extract had good antimicrobial effects on many microorganisms. In this study, the focus was on the antioxidant potential of green tea as well as their antimicrobial effect on different microorganisms. This study was design to assess the broad spectrum antimicrobial effect of green tea, total seven strains of gram-negative bacteria, gram-positive bacteria, and fungal species was used. Aim of the present study was to evaluate the antioxidant and antibacterial potential of green tea polyphenol. Water, hydroalcoholic, alcoholic and ethyl acetate extract were prepared. Antimcrobial effect of all extract were determined by using seven strain of microorganism (*S. aureus, S. epidermidis, Proteus vulgaris, Bacillus subtilis, Escherichia coli* & *Aspergillus Niger, Candida albicans*)

MATERIAL AND METHODS

Green tea leaves was purchase from the local herbal drug distributor and taxonomic evaluation was done from the department of pharmacognosy, PWCOP, Yavatmal. Leaves were dried and grinded to powder.

Extraction. 200 gm of powder extracted by cold maceration method using different solvent water, mixture of water and ethanol (hydroalcoholic),ethanol and ethylacetate. The filtrate was evaporated to dryness under pressure, the process were repeated for three times. Finally, the percentage yield was calculated.

% yield was calculated by the following formula

$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Therotical yield}} \times 100$$

Determination of Total Phenolic Content. Total phenolic content (TPC) of water, hydroalcoholic, ethanolic and ethyl acetate extract, were determined by Folin-Ciocalteau method. The Folin-Ciocalteu method is used for determination of total polyphenolic compound, based on an electron transfer assay, the reduction of MoO_4 + to MoO_3 + that can be measure at 765 nm as change in color from yellow to blue. The principle of Folin–Ciocalteu method is to measure the amount of the substance required to inhibit the oxidation of the reagent, measures the reducing capacity of sample. 1mg/ml of samples were prepared, mix 1ml of sample with 1ml of Folin-Cio-calteau reagent. The mixture was kept for 3 min and then 3 ml of sodium carbonate (7.5%) was added, mixed and kept the mixture for 90 minutes with shaking. Then the absorbance was measured at 760 nm and calibration curve was made by using gallic acid as standard (Singleton *et al.,* 1965; Latif *et al.,* 2013). The experiment was performed in triplicate. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dry wt)

$$T = \frac{C \times V}{M}$$

Where, T - total content of phenolic compounds (mg/g of plant extract),

C- the concentration of gallic acid established from the calibration curve(mg/milliliter),

V: the volume of extract (milliliter) and M is the gram weight of plant extract.

Determination of Antioxidant activity by DPPH Method. Antioxidant activity of water, hydroalcoholic, ethanolic, and ethyl acetate extract were determined by DPPH method. Free radical scavanging activity of different extracts, fractions, and isolated compound was determined by DPPH method. DPPH (1, 1 diphenyl-2-picryl-hydrazyl) is stable free radical which do not become unstable on reaction with antioxidant compound. Antioxidant biomolecules transfers electron or hydrogen atom to colored DPPH molecule, neutralizes its free radical character and convert it to 1,1-diphenyl-2-picryl-hydrazine. This causes change in color intensity of DPPH solution due to free radical scavenging activity of the extracts. Antioxidant Qactivity can be determined by measuring change in the absorbance at 517 nm. DPPH solution (0.3mM) was prepared in ethanol. The concentration of extract, diluted with ethanol were taken as 10ug/ml, 20ug/ml, 30ug/ml, 40ug/ml, 50ug/ml and 1ml of DPPH solution was added, incubated in dark for 30 min at 37°C. After 30min, the absorbance was measured at 517nm. An equal amount of ethanol with DPPH solution was used as a blank (control) (Eugenio *et al.,* 2012; Rahman *et al.,* 2015). The experiment was performed in triplicate.

The percentage of the DPPH radical scavenging was calculated by the following equation

$$\text{Percentage scavenging activity} = \frac{Ac - As}{Ac} \times 100$$

Where Ac- Absorbance of Control

As - Absorbance of Sample

Evaluation of Antimicrobial Activity. Antimicrobial activity of selected water, hydroalcoholic, ethanolic, and ethyl acetate extract was performed by the method given by Suzilla *et al.* (2020). 10 mg of each sample was dissolved in 10 ml of sterile distilled water containing 10% dimethyl sulfoxide (DMSO). This resulted in a stock concentration of 1 mg/ml. From this stock, further dilutions were done. Seven strains of gram negative bacteria, gram positive bacterial and fungal species (*S. aureus, S. epidermidis, Proteus vulgaris, Bacillus subtilis, Escherichia coli* & *Aspergillus Niger, Candida albicans*) were used to assess the antimicrobial potential of the extracts and amoxicillin. Nutrient agar was poured into Petri plates in a laminar airflow cabinet and allowed to solidify. 5 ml of nutrient broth were transferred into each sterile test tube. A loopful two-week-old bacterial, yeast and fungal isolates was inoculated in each 5 ml nutrient broth tube and incubated for 48–72 h. After incubation, the organisms was inoculated using sterile swab sticks and spread on the entire surface of already prepared Mueller-Hinton agar (38 g/l) on culture plates. Wells of 6 mm in diameter were bored into the agar plates using a sterile cork borer. Each concentration of the already diluted samples were transferred into the wells in the volumes of 20 µL, while Amoxicillin 10 mg/L served as a positive control for antibacterial. The plates were incubated at 37°C for 16–18 h (bacteria) and 48–72 h (for fungi). The results were read by measuring the inhibition zone diameter (IZD in mm) across the wells (Daud *et al.,* 2015).

RESULTS AND DISCUSSION

A. Percentage yield

Alcoholic, hydroalcoholic, water and ethyl acetate extract was prepared by cold maceration method and yield was calculated. The % yield of all extract is given in Table 1.

Table 1: Percentage yield of water, alcoholic, hydroalcoholic and ethyl acetate extract of *C. sinensis*.

Name of extract	% yield
Water extract	8.2
Hydro alcoholic extract	14.4
Alcoholic extract	15.2
Ethyl acetate extract	18.5

B. Total Phenolic content

TPC of all extract was determined by Folin-Ciocalteau method. % of phenolic content is given in Table 2.

Table 2: % phenolic content of water extract (GTWE), hydroalcoholic extract (GTHAE), alcoholic extract (GTAE), and ethyl acetate extract of green tea.

Sr. No.	Name of extract	% Phenolic content
1.	GTWE	26.27
2.	GTHAE	28.17
3.	GTAE	27.23
4.	GTEAE	29.32

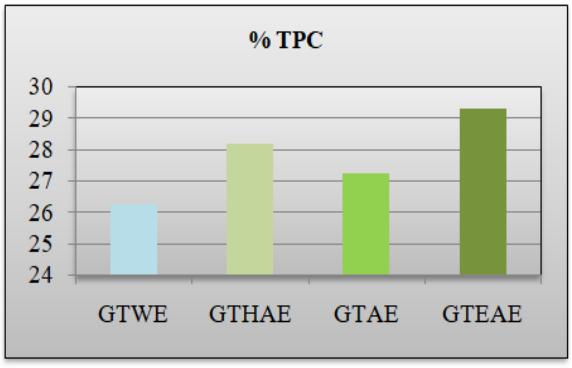

Fig. 1. % phenolic content of water extract(GTWE), hydroalcoholic extract(GTHAE), alcoholic extract (GTAE), and ethyl acetate extract of green tea.

C. Antioxidant Activity

Free radical scavenging effect of all extract was determined at 10, 20, 30, 40, 50 ug/ml. FRE of all extract is given in Table 3.

D. Antimicrobial effect

Minimum inhibitory concentration (MIC) of all extracts was found at 200 µg/ml. The mean diameter of zone of inhibition (ZOI) was found to be dose dependent,

maximum ZOI found at 400 µg/ml against *A. niger*, and *S. epidermidis* than the staphylococcus aureus, and *Bacillus sustilis*. Antimicrobial effect was not found against *Candida albicans, E. coli,* and *P. vulgaris.*

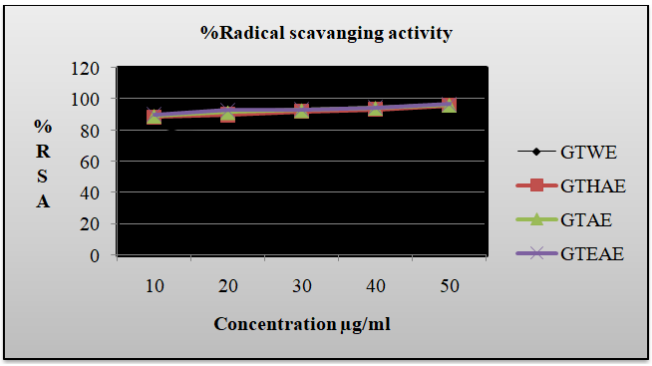

Fig. 2. % Free radical scavenging activity of water extract(GTWE), hydroalcoholic extract(GTHAE), alcoholic extract (GTAE), and ethyl acetate extract(GTEAE) of green tea.

Table 3: Zone of inhibition (ZOI) in mm of water extract (GTWE), hydroalcoholic extract(GTHAE), alcoholic extract (GTAE), ethyl acetate extract (GTEAE) and standard amoxicillin.

Name of sample	*Concentration*	*S. aureus*	*B. subtilis*	*S. epidermidis*	*A. niger*	*C. albicans*	*E. coli*	*P. vulgaris*
GTWE	400 ug/ml	2mm	2mm	4mm	4mm	-	-	-
GTHAE	400 ug/ml	3 mm	2mm	4 mm	5mm	-	-	-
GTAE	400 ug/ml	4 mm	2mm	5mm	6mm	-	-	-
GTEAE	400 ug/ml	4 mm	4mm	6 mm	8mm	-	-	-
Amoxicillin	400 ug/ml	3 mm	5mm	6 mm	8mm	7mm	7mm	4mm

CONCLUSIONS

The total phenolic content result showed that all extracts possess good phenolic content. Free radical scavenging results revealed that all the extracts possess good antioxidant activity. Amongst all extracts, ethyl acetate extract possesses the optimal free radical scavenging effect The antimicrobial effect was determined by using seven strains of microorganisms. Maximum ZOI was found at a concentration of 400 µg/ml. against *A. niger, S. aureus, B. substilis,* and *S. epidermidis*. A more potent antimicrobial effect was found in the alcoholic and ethyl acetate extracts against *A. niger*, and *S. epidermidis* than *B. substilis,* and *S. aureus*. Antimicrobial activity was not found against *E. coli*, *C. albicans*, and *P. vulgaris.* Antioxidant and antimicrobial results reveal that *C. sinensis* could be a potent antioxidant and a novel, safe, and effective antimicrobial agent that could be used in the treatment of many infectious diseases as a chemotherapeutic agent and for topical skin infections.

FUTURE SCOPE

A potent antioxidant and antimicrobial effect was found in all extracts of *C. sinensis.* In the future, it can be used in combination with other antimicrobial phytoconstituents to produce a synergistic antimicrobial effect or with antibiotics, which could minimize antimicrobial resistance. The authors recommended further in vivo studies.

Conflict of Interest. None.

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How to cite this article: Jyotsna Anandrao Saonere, Madhuri A. Channawar, Nitin I. Kochar, Deepak Mohale and Anil V. Chandewar (2023). Evaluation of Antioxidant and Antimicrobial Potential of Herbal Phenolics. *Biological Forum – An International Journal, 15*(2): 1114-1118.