Letter

Phytochemical Screening, Antimicrobial Activity and In Vitro Antioxidant Investigation of Methanolic Extract of Seeds from *Helianthus annuus L.*

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Abstract

This study aimed to evaluate the antioxidant and antimicrobial activity of methanolic extract of seeds from *Helianthus annuus*. The seeds of *Helianthus annuus* was collected, air dried and subjected to methanolic extraction and this extract was screened phytochemically for its chemical constituents. Using standard phytochemical analysis procedures, results revealed the presence of tannins, saponins, flavonoids, carbohydrates, steroids and fixed oils and fats. The methanol extract of seeds of *Helianthus annuus* was subjected to antibacterial and antifungal studies. Antibacterial results are compiled. The seed extract of *Helianthus annuus* showed high sensitivity to *Salmonella typhi*, moderate sensitivity to *Staphyllococcus aureus* and *Vibrio cholera* and less sensitivity to *Bacillus subtilis*. Antifungal results are compiled. The extract of *Helianthus annuus* showed high sensitivity to *Rhizopus stolonifer* and *Aspergillus fumigates*, moderate sensitivity to *Candida albicans* and resistant to *Fusarium oxysporum*. *In-vitro* antioxidant activity of methanolic seed extract of *Helianthus annuus* was determined by DPPH free radical scavenging assay. The methanolic seed extracts of *Helianthus annuus* had shown very significant DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The results concluded that the seeds of *Helianthus annuus* extracts have a potential source of antioxidants of natural origin and have strong potential for use as natural antimicrobials in several applications requiring these properties.

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Key words: *Helianthus annuus*, antimicrobial activity, phytochemical screening, antioxidant activity, DPPH assay

Introduction

Herbal medicines are widely practiced in India where the medicinal plants and their suitable extracts have been extracted and used in the treatment of infections and diseases. *Helianthus annuus* (Asteraceae) an important oilseed crop around the world. *Helianthus annuus* is a widely growing plant which is used traditionally as anti-inflammatory, antimalarial, anti-asthmatic, anti-oxidant, anti-tumor and antimicrobial agent [1]. Besides this, it has been utilized as medicines for thousands of years and now there is a growing demand for plant based medicines, health products, pharmaceuticals and cosmetics.

The Seeds of *Helianthus annuus* have medicinal importance as diuretic, expectorant and also used for cough, throat and lung infections. Healing properties of seed oil of *Helianthus annuus* were used in folk system of therapy for bronchitis, thrombophlebitis, carbuncles, catarrh, blindness, sinusitis, diarrhea, dysentery, dysuria, rheumatism, hemorrhoids, eye infections, fever, toothache, laryngitis, menorrhagia, pleuritis, scorpion stings, snakebite, inflammation, urogenital ailments, splenitis, aroma therapy, leg ulcer, whitlow and wounds [2]. The seeds and flowers of this plant are febrifuge, stomachic and provide excellent source of nutrition and also reported that it was used in the treatment of many pulmonary disorders. Decoction of roots of *Helianthus annuus* has been used as a warm wash on rheumatic aches and pains [3]. Sunflower seeds are often eaten raw or preferred to be eaten roasted because they are rich source of protein and Vitamins B, D, E and K. Research studies have shown that it can prevent us from cancer and many more harmful diseases [1].

Medicinal plants contain various phytochemicals that are very important for human life for the treatment of various diseases. Antioxidants present in the plants play an essential role in protecting the cells and tissues

against damage caused by reactive oxygen species. Plants containing flavonoids have been reported to possess strong antioxidant properties [4]. Previous studies have shown that *Helianthus annuus* possess antimicrobial activity and antioxidant capacity [5]. However, there is insufficient information regarding the antimicrobial and antioxidant activities of methanolic extract of seeds from *Helianthus annuus*. The intention of the present study was mainly to comprehend the phytochemical screening, antimicrobial and antioxidant activities of crude extracts of seeds from *Helianthus annuus* as part of the exploration of new and novel bio-active compounds.

Materials and Methods

Collection of plant material and identification

Seeds of *Helianthus annuus* were collected from Anna medicinal farm, Chennai. The identification of plant material was done on the data present in previous literature and it was authenticated by Dr. Seethalakshmi of Lifeteck Research Centre. The seeds were sorted, cleaned and air dried at room temperature for 2 weeks and then powdered to prepare the extract.

Preparation of methanolic extract

About 10g of air dried powder of seeds of *Helianthus annuus* was mixed with 100 mL methanol in a conical flask and kept in rotary shaker at 150 rpm for 24h. After 24h, it was filtered and the solvent was evaporated to make the final volume to be one-fourth of the initial volume. It was stored at 4°C for further studies. The extract was dissolved in Dimethyl sulphoxide (DMSO) to a concentration of 100mg/mL [5].

Preliminary Phytochemical analysis:

The obtained seed extract of *Helianthus annuus* were screened for the presence of biologically active compounds like glycosides, alkaloids, tannins, flavonoids, saponins and steroids. Qualitative phytochemical analysis of the seed extract was undertaken using standard qualitative methods as described by various authors [6, 7].

Microrganisms used

The following bacterial and fungal clinical isolates were obtained from stock culture of selected pathological strains, which were preserved at the Lifetck Research Centre. *(Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Rhizopus stolonifer, Aspergillus fumigatus, Fuserium oxisporium, Candida albicans)*

Assay of antibacterial activity

Preparation of active bacterial suspension:

Stock bacterial cultures were maintained at 4°C on nutrient agar slants. Active cultures for experiments was prepared by transferring a loopful of stock culture to 10mL of nutrient broth and incubating aerobically at 37°C for 24h for bacterial proliferation.

Determination of antibacterial activity of plant extracts

Muller Hinton agar solid media was used for culturing of bacteria. Agar diffusion assay was carried out to check the antibacterial activity as described by [8]. The medium was sterilized in autoclave for 30 mts and then allowed to cool but not solidify. They are then transferred to 4 different petri dishes and then made to solidify naturally. Then freshly prepared cotton swabs which are sterilized are used to swab the developed bacterial culture onto the solidified medium. Whatmann No 3 filter paper discs were impregnated with 20µl of different concentration of plant extract, kept onto the petri plate and incubated at 37°C for 24h. [9]. Ampicillin (10µg/mL) was used as the control.

Assay of antifungal activity

Preparation of working fungal cultures

Antifungal activity of the extracts was determined by antifungal susceptibility test. Potato dextrose broth is inoculated with stock fungal cultures. Then the tubes were kept in an orbital shaker (200 rpm) for up to 4 days at 30°C.

Preparation of medium

Potato dextrose agar (3.9g) is mixed with 100mL of distilled water. It is then sterilized in autoclave for 30 mts and then allowed to cool but not solidify. They are then transferred to 4 different Petri dishes and then made to solidify naturally. Then freshly prepared cotton swabs which are sterilized are used to swab the developed fungal culture onto the solidified medium. Whatmann No 3 filter paper discs were impregnated with 20µl of different concentration of plant extract, kept onto the petri plate and incubated at 37°C for 24 h. The growth of inhibition was observed for 2-4 days. Amphotericin B (25µg/mL) was used as the control [10].

Antioxidant Assay

The antioxidant activity of seed extract was determined by *in vitro* method (DPPH free radical scavenging assay). The free radical scavenging capacity of the methanolic extract of *Helianthus annuus* was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Methanol extract of *Helianthus annuus* was mixed with 95% methanol to prepare the stock solution (10mg/100mL). The concentration of this *Helianthus annuus* methanolic extracts solution was

10mg /100mL or 100µg/mL. From stock solution 2mL, 4mL, 6mL, 8mL & 10mL of this solution were taken in five test tubes & by serial dilution with same solvent were made the final volume of each test tube up to 10mL whose concentration was then 20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL & 100µg/mL respectively. Freshly prepared DPPH solution (0.004%w/v) was added in each of these test tubes containing *Helianthus annuus* methanolic *extracts* (20 µg/, 40µg/mL, 60µg/mL, 80µg/mL, 100µg/mL) and after 10 min, the absorbance was taken at 517 nm using a UV–visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10 mg/100mL or 100µg/mL) of methanolic extracts of *Helianthus annuus*. Control sample was prepared without adding any extract and ascorbic acid. 95% methanol was used as blank [4].

(The Scavenging activity of DPPH (%) was calculated by using the following equation –
% DPPH radical scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100

Results and Discussion

The results of the phytochemical analysis showed that carbohydrates, alkaloids, flavonoids, tannins, saponins, phytosterol, steroids, fixed oils and fats are present in the seeds of *Helianthus annuus* extract (**table 1**). The varied phytochemical constituents present in the seed extract such as flavonoids, alkaloids, saponins were reported to possess biological activity against microbes [8, 11].

Depending upon the measured values of inhibition the seed extract of *Helianthus annuus* (50µg/mL) showed high sensitivity to *Salmonella typhi* (1.5cm), moderate sensitivity to *Staphylococcus aureus* (1.2cm), *Vibrio cholera* (1.1cm) and very less sensitivity to *Bacillus subtilis* (0.7cm). The antibacterial activities are comparable with the reference drug *Ampicillin* (10µg/mL). The antifungal activity of *Helianthus annuus* plant extract was assayed by agar well diffusion method. Methanol seed extracts (50µg/mL) showed high activity against *Aspergillus fumigates* (1.3cm), *Rhizopus stolonifer* (1.2cm), moderate activity against *candida albicans* (1.0cm) and less activity against *Fusarium oxysporum* (0.5cm). The antibacterial and antifungal activity of *Helianthus annuus* as recorded in this study may therefore due to the presence of flavonoids, alkaloids, saponins and tannins in the extract. These phytoconstituents particularly tannins and flavonoids are proven to induce an important antimicrobial activity due to their possession of ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and so forth [13].

Table 1 Preliminary Phytochemical analysis of methanolic extract of seeds of *Helianthus annuus* (+ Present, - Not Present)

Phytoconstituents	Test	Inference
Carbohydrates	Molisch test	+
Starch	Iodine test	-
Alkaloids	Dragendorff's test Wagner's test Mayer's test	+
Flavonoids	Alkaline reagent test Shinoda's test	+
Tannins	Ferric chloride test Lead acetate test	+
Glycosides	Legal's test Borntrager's test	-
Saponins	Lead acetate test	+
Phytosterol	Salkowski's test Libermann Burchard test	+
Steroids and Triterpenoids	Libermann Burchard Noller test	+
Protein	Ninhydrin's test	-
Fixed oils and fats	Spot test	+

Table 2 In vitro Antibacterial activity of Helianthus annuus seed extract

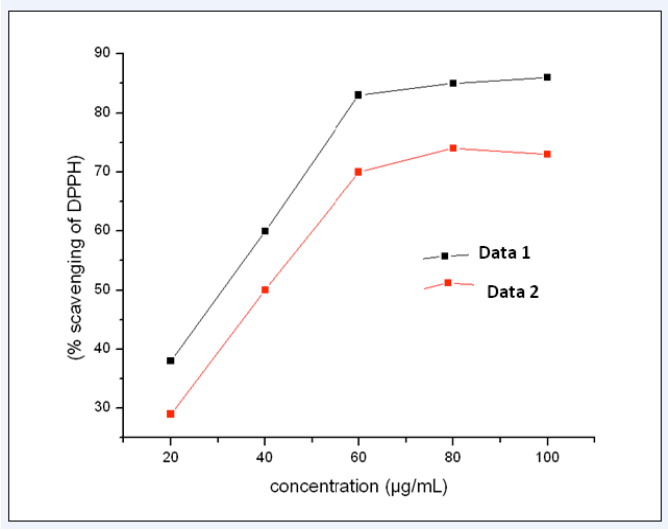
Strains of Microorganisms	Zone of inhibition (cm)			Ampicill-in (control)
(20µl)	50µg / mL	25µg / mL	10µg / mL	10µg/ mL
Salmonella typhi	1.5	1.3	1.0	1.6
Bacillus subtilis	0.7	0.6	0.5	1.0
Staphylococcus aureus	1.2	0.9	0.8	1.7
Vibrio cholerae	1.1	0.9	0.8	1.4

Previous reports showed that Ocimum basilicum extract contains flavonoids and it possesses antibacterial activity [12]. Antibacterial and antifungal activity of seeds extract of *Helianthus annuus* were studied by measuring the zone of inhibition formed around the disc and the results are given in table 2 and 3.

Table 3 In vitro Antifungal activity of Helianthus annuus seed extract

Strains of Microorganism (20µL)	**Zone of inhibition (cm)**			**Amphoter-cin (control)** (25µg/ mL)
	50µg /mL	25µg /mL	10µg/ mL	
Fuserium oxisporium	0.5	0.2	-	0.6
Rhizopus stolonifer	1.2	0.9	0.5	1.6
Candida albicans	1.0	0.8	0.6	1.4
Aspergillus fumigatus	1.3	0.9	0.6	1.7

DPPH radical scavenging activity of seed extract of *Helianthus annuus* and ascorbic acid are presented in **figure 1**.



Data 1 - % inhibition by Helianthus annuus

Data 2 - % inhibition by Ascorbic acid

Figure 1 DPPH radical scavenging activity of methanolic seed extract of Helianthus annuus added to methanolic solution of DPPH and radical scavenging activity was measured as 517 nm as compared to standard Ascorbic acid.

The antioxidant activity of the methanol extracts from seeds of *Helianthus annuus* were investigated by using DPPH scavenging assay. Methanolic seed extracts of *Helianthus annuus* has got profound antioxidant activity when compared to the reference standard antioxidant Ascorbic acid. The DPPH radical scavenging assay is based on the ability of DPPH, (stable free radical) to decolorize in the presence of antioxidants. The DPPH radical possess an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Compared activity of Helianthus annuus extract and standard ascorbic acid were shown in **Figure 1**.The methanol seed extract of *Helianthus annuus* exhibited a significant inhibition of DPPH activity. It has been proven that reactive oxygen species play an important role in various pathophysiological conditions and endogenous defense mechanisms [14]. By increasing the antioxidant reserve of an organism, the oxidative stress can be reduced [15]. The plants are considered as good sources of potential antioxidants for various medicinal uses and determination of these antioxidant compounds will help to develop new drug compositions.

Conclusion

In conclusion *Helianthus annuus* seed extract possess a broad spectrum of activity against bacterial and fungal strains responsible for infections. Its potential application in the treatment of bacterial and fungal infection would therefore be promising. Methanol extracts of seeds of *Helianthus annuus* were found to have recognizable per oxidation inhibition activity. Owing to these antioxidant properties, the above plant can be considered as natural source of dietary antioxidants. Further work is required in order to isolate the active constituents of the plant responsible for the antimicrobial activity and antioxidant activity.

Acknowledgement

The authors are grateful to the Lifeteck Research Centre (Chennai, India) for providing necessary laboratory facilities. Thanks are also to Dr. Seethalakshmi, Lifeteck Research Centre for her constant encouragement and suggestions while doing the experiments.

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Received : 14th June, 2012

Revised : 2nd August, 2012

Online : 5th August, 2012