

## Phytochemical analysis, antioxidant and antibacterial properties of *Opuntia Ficus-indica* (L.) mill. against the wound infecting bacteria

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### Abstract

Oxidative stress is initially generated by free radicals which makes electrons to paired with biological macromolecules in cells of healthy humans and induce DNA and protein damage. Infectious diseases are still a major threat to the public health despite of enormous development in the medical field. Nowadays various synthetic drugs became resistant to various infectious diseases. Therefore, various phytochemicals in medicinal plants are in use for the treatment of several oxidative stress related disease. In the present study, Cactus Pear was selected to analyze the phytoconstituents using aqueous extract. Phytochemicals like phenols, flavonoids, alkaloids, tannin, saponin and glycosides was observed in aqueous extract of Cactus pears. Total flavonoids and phenolics content was also evaluated by aluminum chloride method and Folin's Ciocalteu methods respectively. Antioxidant and radical scavenging efficiency was evaluated by reducing power assay and hydroxyl radical scavenging assay. An excellent reducing potential and radical scavenging activity was observed in aqueous extract of Cactus pear. Finally efficiency of Cactus pear against the growth of *Methicillin Resistant Staphylococcus aureus* (MRSA) from clinical isolates was evaluated. Aqueous extract of *Opuntia ficus-indica* exhibited excellent efficiency in *anti-staphylococcal* activity. Hence, based on the above studies, we could confirm that, *O. ficus-indica* could be used as a potential drug for the treatment of oxidative stress related diseases.

**Keywords:** *Opuntia ficus-indica*, phytochemicals, total phenolics, flavonoids, radical scavenging activity, MRSA

### Introduction

*Methicillin Resistant Staphylococcus aureus* (MRSA), a notorious gram positive bacterium is a leading cause of various human infections in skin, bones, joint and heart valves. MRSA can easily get attaches to the host tissue and induces serious disease with the help of multiple virulence factors [1]. Oxidative stress is a major contributor for the patho-physiological conditions including cardiovascular dysfunctions, inflammation, carcinogenesis and neuro generative diseases [2]. Natural substances contains prominent source for the treatment of the several infections and also reduces the formation of free radicals with the help of radical scavenging biomolecules [3]. About 75% of drugs are obtained from the natural products for the treatment of various infectious diseases and oxidative stress related diseases [4]. Since ancient age, traditional and folk medicinal system uses, plant products as a medicinal source for the treatment of various infectious diseases. In recent years, various researchers exploring about medicinal properties of herbal plants against various diseases and they proved that this plants could be a major source for the drug discovery and development [5, 7]. *Opuntia ficus-indica* belongs to Cactaceae family which is naturally originated in arid and semi-arid regions of Mexico, Latin America and South Africa [8, 9]. Cactaceae reported to contain about 130 genera and 1500 species. It produces cactus fruit, otherwise called as *Cactus ficus* which is purple in colour, fleshy nature and oval in shape. It is commonly named as Chapathi Balli in Tamil Nadu [10]. It produces sweet nutritionally rich edible fruit, tender cladodes of cactus which is used as a fresh green vegetable and salad. They are used to prepare products like jam, squash, wine, pickle, body lotion,

shampoo and creams [11]. It has been traditionally used as folk medicine because of its role in treating number of diseases and conditions like anti-inflammatory [12], hypoglycemic [13]; inhibition of stomach ulcer [14], neuroprotective effect [15]. Antioxidant efficiency of cactus plant made them to be used to treat diabetes, burns, brochial, edema, asthma and indigestion in all over the world [16]. Finally it is reported to possess certain pharmacological activities like anti-ulcer [17]; anti-inflammatory [12, 18]; neuro-protective [15]; anti-cancer [19]; anti-viral [20]; alcohol hangover [21]; anti-diabetic [9]; hepato-protective [22]; antioxidant [23]; anti-viral [24]; anti-bacterial [25] activities. This plant is frequently used by the local population to treat several infectious diseases and known to possess several biological activities. Nowadays several researches have been going in order to discover the pharmacological properties of *Opuntia* plant species.

Hence the intention of present study to put forward for explore the yield of extract, screening of phytoconstituents, quantification of total phenolics and flavonoids from aqueous extracts of Cactus ficus and also to analyze their anti-staphylococcal efficiency against *Methicillin Resistant Staphylococcus aureus* (MRSA) and antioxidant activities.

### Materials and Methods

#### Collection of Plant Material

*Opuntia ficus-indica* was collected from Perumanallur of Tirupur district, Tamil Nadu, India, during the period of May and June -2019. They were washed in tap water followed by distilled water and dried in shadow. The dried pods were grounded as course powder in electrical blender and stored in air tight container for future use.

### Extraction of Plant Material

About 100g of dried pods of cactus pear was suspended with 500 ml of distilled water and incubated in shaker at 190-220 rpm for 24 hrs by cold percolation method. Then filtered through layers of muslin cloth and dried [26].

### Phytochemical Analysis

The preliminary phytochemical screening of aqueous extract of *O. ficus-indica* was conducted to identify the presence of bioactive compounds like alkaloids, flavonoids, tannins, phenols, saponins, tannins, terpenoids, glycosides and steroids [27].

The total phenolics content in aqueous extract of *O. ficus-indica* was determined by spectrophotometric method [28]. About 100 µl of aqueous extract of *O. ficus-indica* was added to the 2 ml sodium carbonate (2%) as prepared freshly. About 2 ml of Folin ciocalteu reagent was added to the mixture and it was incubated at room temperature for 30 minutes. Then the optical density was measured spectrophotometrically at 750 nm against blank with gallic acid as a positive control.

Amount of total flavonoids content was determined by aluminium chloride method spectrophotometrically [29]. About 500 µl of aqueous extract of *O. ficus-indica* was added to 2 ml of distilled water followed by 1.5 ml of sodium nitrite (1.5%). After 6 minutes of incubation, 2 ml aluminium chloride (10%) was added to it and incubated for 5 minutes. Then, 2 ml of 4 % sodium hydroxide was added to it. Then the mixture was incubated for 15 minutes at room temperature. Absorbance of the mixture was measured spectrophotometrically at 510 nm against blank with quercetin as a positive control.

### Isolation and Identification of Wound Sample

Isolates of bacterial culture was collected from septic wound samples at Perunthurai Medical College, Erode. Collected samples were inoculated on mannitol salt agar and mackonkey agar and incubated at respective temperature.

### Antibacterial Activity

Antibacterial activity of aqueous extract of *O. ficus-indica* was evaluated against *Methicillin Resistant Staphylococcus aureus* (MRSA) by agar well diffusion method. About 20 ml of Muller Hinton agar was poured in to petri dish and allowed to cool. Broth culture of MRSA was swabbed over the MHA. Then 5 well were poured into the plate with well puncher. Different concentration of aqueous extract of *O. ficus-indica* added into well with positive, negative control [30].

### Minimum Inhibition Concentration (MIC)

MIC of aqueous extract of plant and green synthesized AgNPs were done using the method described in the guideline of CLSI (2012). MIC test was performed in 96-well round bottom microtiter plate using standard broth microdilution method. The bacterial inoculums were adjusted to the concentration of  $10^6$  CFU/mL. For the MIC test, 100 µL of aqueous extract of *O. ficus-indica* (500

µg/mL) was added and diluted twofold with the bacterial inoculums in 100 µL of MHB started from column 12 to column 3. Column 12 of the microtiter plate contained the highest concentration of Cactus Pear aqueous extract while column 3 contained the lowest concentration. Column 1 served as negative control (only medium) and the column 2 served as positive control (medium and bacterial inoculums). Each well of the microtiter plate was added with 30 µL of resazurin solution and incubated at 37°C for 24 h. Any color changes were observed. Blue/purple color indicated no bacterial growth while pink/colorless indicated bacterial growth. The MIC value was taken at the lowest concentration of antibacterial agents that inhibits the growth of bacteria (color remained in blue).

### Antioxidant Activity

Antioxidant activity of *O. ficus-indica* was carried out by reducing power assay and hydrogen peroxide radical scavenging activity

The reducing activity of aqueous extract of *O. ficus-indica* was determined [31]. 1ml of extract (20-100 µg/ml) was prepared in distilled water and mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 ml of 10% trichloroacetic acid was then added to the mixture and centrifuged at 3000 rpm for 10 min. 1 ml of aliquot of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl<sub>3</sub> (10%) and absorbance was measured at 700 nm.

To the 1 ml of different concentrations (20-100 µg/ml) of the aqueous extracts of *O. ficus-indica* and hydrogen peroxide prepared with phosphate buffer (pH 7.4) was added in the volume of 0.6 ml. then the mixture was kept at room temperature for 10 minutes and the absorbance was measured at 230 nm using UV-visible spectrophotometer.

Inhibition (%) =  $\frac{\text{control OD} - \text{sample OD}}{\text{Control OD}} \times 100$ .

### Result and Discussion

The yield percentage of extract is a measure of the efficiency of solvents to extract specific bioactive compounds from the original materials and it was defined as the amount of extract recovered in mass compared with the initial amount of whole plant. The yield per cent of leaf extracts with distilled water. The maximum yield was obtained in aqueous extract of *O. ficus-indica* (8.498 %). The information gained on the percent yields of crude extracts were used for standardizing dosage rates of fine powder preparations of the plant materials. The amount of crude extract contained in a known weight of fine powder of plant materials can then be calculated. This agrees with findings by Ejobi and Olila 2004; Olila *et al.* (2007) [32, 33]. Yield extraction is an indicator for the effect of extraction conditions. Extraction of *O. ficus-indica* with distilled water provided yield of about 8.498%. This could be due to the high polar nature of water.

## Phytochemical Screening of Aqueous Extract of *O. Ficus-Indica*

As bioactive components in plant plays an important role in defense against prey, micro-organism, oxidative stress and interspecific protection. These bioactive components in plant extracts has been used a drug for the various ailments. Hence, phytochemical screening acts as a primary procedure to identify the phytoconstituents in the plants [34]. Phytochemical screening of *O. ficus-indica* of aqueous

extract revealed the presence of constituents like alkaloids, flavonoids, phenolics, tannins, saponins, carbohydrates, terpenoids and proteins. Specifically higher concentration of alkaloids, flavonoids, phenolics, carbohydrates and terpenoids was observed. Moderate concentration of tannins and saponins was observed. Mild concentration of proteins was observed. Results of phytochemical screening were tabulated in Table 1.

**Table 1:** Phytochemical screening of aqueous extract of *Optunia ficus-indica*

Phytoconstituents	Assays	Indication	Aqueous extract of <i>Optunia ficus Indica</i>
Alkaloids	Dragendroff Wagner	Yellow precipitate Reddish brown precipitate	+++
Flavonoids	Shinoda Lead acetate	Pink Yellow	+++
Phenolics	Ferric chloride	Dark green	+++
Tannins	Lead acetate	White precipitate	++
Saponins	Foam test Honey comb	Foam Froth	++
Carbohydrates	Molisch's test Fehlings's test	Violet ring Red precipitate	+++
Terpenoids	Salkowski	Red and yellow green fluorescence	+++
Proteins	Biuret Ninhydrin	Violet Purple	+

+: Low, ++: Mild, +++: Strong, -: Absence

Aqueous extract of *O. ficus-indica* exhibited orange precipitate and reddish brown precipitate by dragendroff and wagners reagent assay. This confirmed the presence of alkaloids in aqueous extract of *O. ficus-indica*. Pink and yellow colour was observed in aqueous extract of *O. ficus-indica* by Shinoda and lead acetate test. This confirmed the presence of flavonoids in aqueous extract of *O. ficus-indica*. Ferric chloride test revealed dark green colour which confirmed the presence of phenolics in aqueous extract of *O. ficus-indica*. A white precipitate was observed in the lead acetate test which confirmed the presence of tannin in aqueous extract of *O. ficus-indica*. Foam and honey comb test revealed the presence of saponin in aqueous extract of *O. ficus-indica* by forming foam and froth. Analysis of carbohydrate was done by Molisch and Fehling's test which forms violet ring and red precipitate revealed the presence of carbohydrates in aqueous extract of *O. ficus-indica*. A red and yellow fluorescence was observed in salkowski test for the analysis of saponin which confirmed the presence of saponins in aqueous extract of *O. ficus-indica*. Finally presence of protein in aqueous extract of *O. ficus-indica* was revealed by the formation of violet and purple color by biuret and ninhydrin test.

Alkaloids, tannins, saponin, terpenoids and carbohydrate are well known due to its biological activity of these compounds. Aqueous extract of *O. ficus-indica* revealed the presence of these compounds ranges from mild, moderate and high concentration. Phenolics derivatives like flavonoids, tannins and phenolics acid were reported to exhibit antibacterial especially against *staphylococcus aureus*, antioxidant, chemopreventive properties [35, 37].

### Quantification of Total Phenolics and Flavonoid Content

The total phenolics and flavonoids content in the aqueous extract of *O. ficus-indica* were found to be  $75.34 \pm 0.35$  mg/GAE and  $37.42 \pm 0.67$  mg/QE respectively. Plants contain diverse group of phenolics compound like phenolics, flavonoids and their derivatives. All these

phenolics derivatives has vast application because of their physiological functions like free radical scavenging, anti-mutagenic, anticarcinogenic and anti-inflammatory functions [38].

Phenolics derivatives are play an important role in antioxidant and antimicrobial activity. The antioxidant activity of phenolics derivatives could be due to their redox potential which makes them acts as reducing agent, hydrogen donar, singlet oxygen quenchers and potential metal chelators. It also has an ability to delay the pro-oxidative effect of protein, DNA and lipids through the formation of stable radical [39]. The phenolics compounds acts on cell membrane and cell wall of microbes in two different ways. They form hydrogen bonding with membrane protein of bacteria through their hydroxyl groups. This in turn results in the changes of membrane permeability and causes cell destruction. It can also penetrate into the bacterial proteins and coagulate cell content [40, 41]. In this study high level of phenolics content was observed in the aqueous extract of *O. ficus-indica*.

### Anti-Staphylococcal Activity

*Methicillin Resistant Staphylococcus aureus* (MRSA) causes bacteremia, pneumonia, osteomyelitis, endocarditis, sepsis and toxic shock syndrome [42]. The antibacterial activity of aqueous extract of *O. ficus-indica* was evaluated using agar well diffusion method for their anti-bacterial properties. The inhibitory effect of aqueous extract of *O. ficus-indica* was observed at concentration of 50 mg/ml & 100 mg/ml. Inhibitory concentration of the extracts of *O. ficus-indica* was mentioned in Table 2. At concentration of 100 mg/ml aqueous extract of *O. ficus-indica* exhibited potential anti-staphylococcal activity against 5 different clinical isolated namely MRSA1, MRSA20, MRSA7, MRSA2 and MRSA5 with zone of inhibition  $30.5 \pm 0.70$ ,  $27.85 \pm 0.21$ ,  $26.85 \pm 0.21$ ,  $25.25 \pm 0.35$  and  $24.45 \pm 0.63$  mm respectively.

**Table 2:** Antibacterial activity of aqueous extracts of *Optunia ficus-indica* against *Methicillin Resistant Staphylococcus aureus* strains by well-diffusion method

Isolates	Concentrations (mg/ml) (Zone of Inhibition in mm)	
	50 mg	100 mg
<i>S. aureus</i> 1	27.4±0.56	30.5±0.70
<i>S. aureus</i> 2	24.45±0.63	25.25±0.35
<i>S. aureus</i> 5	20.75±0.35	24.45±0.63
<i>S. aureus</i> 7	28.5±0.70	26.85±0.21

Minimum inhibitory concentration of aqueous extract of *O. ficus-indica* was employed by agar well diffusion method to evaluate bacteriostatic effect. The concentrations of aqueous extract of *O. ficus-indica* were reported in Table 3. Inhibitory effect of aqueous extract of *O. ficus-indica* was stated at 1.95 mg/ml with the zone of inhibition 11.54±1.45, 10.32±1.12, 9.14±1.23, 8.34±0.17 and 7.43±2.13, against clinical isolates of MRSA1, MRSA20, MRSA7, MRSA2 and MRSA5 respectively. Bargougi *et al.*, (2019) reported *anti-staphylococcal* effect using ethyl acetate and methanolic extracts of *O. ficus-indica* from 4 different cultivars [43]. Methanolic extracts of four different cultivars namely *Ain Amara*, *Lengissima*, *Ain Jemma* and *Sanguinea* inhibited the growth of *Staphylococcal aureus* namely

27±2.47, 24±1.16, 29±2.06, 25±1.66 respectively. Similarly Ethyl acetate extracts of four different cultivars namely *Ain Amara*, *Lengissima*, *Ain Jemma* and *Sanguinea* inhibited the growth of *Staphylococcal aureus* namely 14±1.77, 12±0.98, 11±1.37, 13±1.47 respectively. Variations in results could be due to the variations in their methods of extraction, solvent used for extraction, chemical constituents of plants and bacterial strain used. Investigation on the potential of plant extracts and their effective compounds as anti-microbial agents to control growth of food borne and spoilage bacteria was carried out by several researchers. Some scientists have also reported that anti-microbial components of plant extracts like terpenoids, alkaloids and phenolics derivatives interact with enzymes and proteins of microbial cell membrane which causes its disruption to spread protons towards cell exterior which in turn induces cell death or may inhibit enzymes which is necessary for amino acids synthesis [44, 45].

Tiwari *et al.*, (2009) [46] and Friedman *et al.*, (2004) [47] reported that herbal plants have hydrophobic character which enable them to react with proteins of microbial cell membrane and mitochondria which disturbs their structure and changing their permeability.

**Table 3:** Minimum Inhibition Concentration of aqueous extract of *Optunia ficus-indica*

Isolates	Concentration in mg/ml of aqueous extract of <i>Optunia ficus-indica</i>											
	500	250	125	62.5	31.25	15.62	7.81	3.90	1.95	0.97	0.48	0.24
MRSA1	-	-	-	-	-	-	-	-	+	+	+	+
MRSA2	-	-	-	-	-	-	-	-	+	+	+	+
MRSA5	-	-	-	-	-	-	-	-	+	+	+	+
MRSA7	-	-	-	-	-	-	-	-	+	+	+	+
MRSA20	-	-	-	-	-	-	-	-	+	+	+	+

“+” = indicates the presence of organisms; “-” = indicates the absence of organisms

### Antioxidant Activity of *O. ficus-indica*

Antioxidant activity of aqueous extract of *O. ficus-indica* was analyzed by its reducing potential and radical scavenging ability. Reducing efficiency of extracts plays significant influence on the antioxidant potential of plant extracts. The reducing potential of aqueous extract of *O. ficus-indica* was evaluated by ferrous reduction assay. In this assay colour of test solution changes from yellow to green and blue depending on the concentration of aqueous extract of *Optunia ficus-indica* and also based on the reducing potential of the aqueous extract of *O. ficus-indica*. Antioxidants in the plant extracts reduces the Fe<sup>3+</sup> ferrocyanide complex to Fe<sup>2+</sup> which was monitored by the formation of Perl's Prussian blue at 700 nm [48].

In the present study, aqueous extract of *O. ficus-indica* containing antioxidant (reductant) induces the reduction of Fe<sup>3+</sup> ferrocyanide complex to Fe<sup>2+</sup> by donating an electron at 700 nm. Aqueous extract of *O. ficus-indica* exhibited a dose dependent reducing potential ranges from 20-100 mg/ml (Figure 1). Increase in the concentration of aqueous extract of *O. ficus-indica* increases the reducing potential of antioxidants (22.32 – 85.89 mg/ml). Hence aqueous extract of *O. ficus-indica* was found possesses efficient antioxidant

potential by expressing excellent reducing potential at 100 mg/ml. Plants are rich in phenolics which have an ability to scavenge free radical and antioxidant activity due to the presence of hydroxyl groups [49].

In living systems, uninhibited accumulation of hydrogen peroxide leads to the development of oxygen free radicals like hydroxyl radicals which causes huge damage to cell membrane. Consequences of such reactions are observed in various diseases like atherosclerosis, cancer and neurological disorders. The hydrogen peroxide radical scavenging activity of extracts was evaluated spectrometrically at 230nm. Hydrogen peroxide radical scavenging activity of aqueous extract of *O. ficus-indica* was found to be concentration dependent and this range is 19.32 – 83.56 µg/ml (Figure 2). With increase in the concentration of aqueous extract of *O. ficus-indica*, hydroxyl radical scavenging activity of extract increases. The inhibition percentage of aqueous extract of *O. ficus-indica* was found to be 83.56% at 100 mg/ml. Our results indicated that aqueous extract of *O. ficus-indica* was found to be a potent radical scavenger. Phenolics are the efficient radical scavenger and metal chelator because of the redox properties, hydrogen donor and singlet oxygen quencher [50].

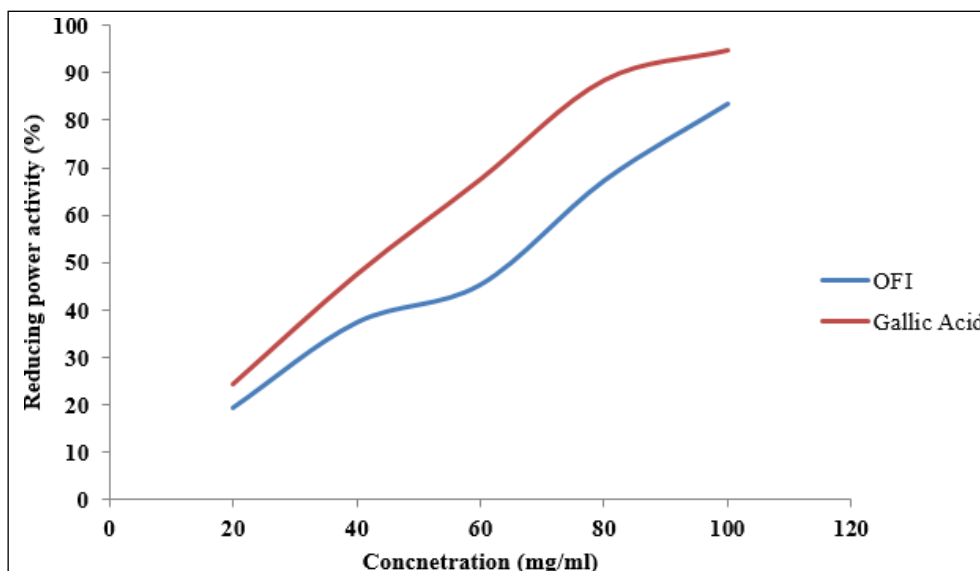


Fig 1: Reducing power of aqueous extract of *Optunia ficus-indica*

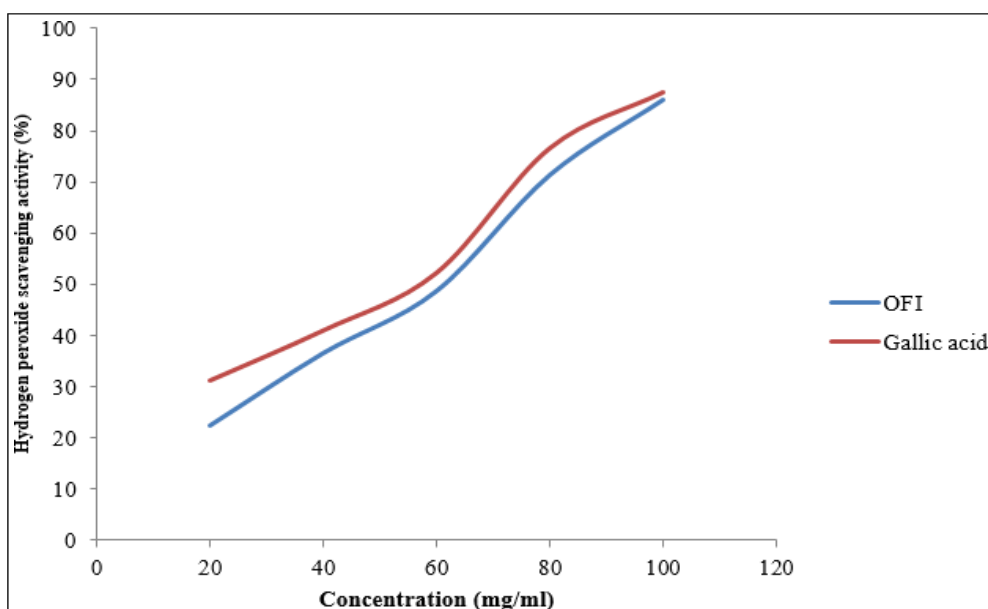


Fig 2: Hydrogen peroxide scavenging activity of aqueous extract *Optunia ficus-indica*

### Conclusion

In conclusion, *O. ficus-indica* extracted with distilled water and maximum yield percentage was obtained due to its high polar nature. Phytochemical screening of aqueous extract of *O. ficus-indica* revealed the presence of phenolics, flavonoids, tannin, saponin, carbohydrates, alkaloids and proteins. Total phenolics and flavonoids in aqueous extract of *O. ficus-indica* reported to be higher in concentrations. All these phytoconstituents reported to possess various pharmacological activities such as antibacterial, antioxidant and chemo-preventive activities. Hence, *Anti-Staphylococcal* and antioxidant activity of aqueous extract of *O. ficus-indica* could be due to the presence of these bioactive compounds. In conclusion, *O. ficus-indica* could be used as a potent drug for various disease treatments.

### Conflict of interest

There is no conflict of interest

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None

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