Anti-urease and antioxidant activity on selected medicinal plants: *Nigella sativa, Ricinus communis, Ocimum sanctum and Curcuma longa*

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Abstract

Nigella sativa, Ricinus communis, Ocimum sanctum,Curcuma longa, a popular medicinal plant has been used since ancient times to prevent and treat diseases and enhance general health and well-being. The current study was focused to the evaluation of antioxidant and anti-urease activity.The antioxidant activity of all extracts (NS, RC, OS and CL) was assessed through DPPH assay method using BHA as a standard drug. For the evaluation of anti- urease activity Indophenols method was adopted which quantify the ammonia and the enzyme activity, that determined by measuring its absorbance.The results indicated that the free radical power of CL (29.6±0.62) was stronger than other extracts i.e., NS (39.5±0.49), RC (42.1±0.56) when compared to BHA (44.2±0.24). Less activity was shown by OS extract (65.7±0.15).The highest anti urease activity was shown by CL extract (29.8±0.39) when compared to the standard Thiourea (22.4±0.29). Followed by OS (45.7±0.42) extract, whereas NS and RC extracts (55.8±0.13, 49.9 ± 0.98) were moderately less active. On the bases of results, *Curcuma longa* extract showed significant anti-urease activity.

The recent study has offered a new approach to exploit antioxidant and anti-urease prospects of these medicinal plants for therapeutic perseverance in new cure of several diseases.

Keywords

Anti-urease, antioxidant, *Curcuma longa, Nigella sativa, Ocimum sanctum, Ricinus communis*

1. INRODUCTION

Medicinal plants have been used since ancient times to prevent and treat diseases for the improvement of general health .The effect of the whole herb is different from any selected part of the plant or their extracts. The constituents present in the medicinal plants may be efficacious in the treatment of different organs/systems. Natural origin medicines may exhibit adverse effects and

interact with others over the counter prescription medicines. Therefore, herbal medicines should be taken according to competent practitioner prescription. Plants have the positive influence and potential to enhance overall well-being and quality of life of human beings. Extensive research works are in progress to authenticate and standardize the plants and medicinal products derived from them with existing documented, established ethno-pharmacological claimed efficacy and safety (Kumar *et al*. 2015; Da-Yong and Ting-Ren, 2019).

Urease enzyme contains protein component that is present in various micro-organisms (bacteria, fungi, yeast) and plants for maintaining their vitality but may be responsible for causing variable pathologies (gastritis, urolithiasis, pyelonephritis and cancer) in humans. Various plants contain constituents possessing potent anti-urease activity equivalent or better than synthetic anti-urease agents. Research is in progress to identify urease inhibitors from plants source with efficacy and least toxic effects (Amin *et al*. 2013; Bai *et al*. 2013). The following plants are being explored in current study to identify potent anti-urease activity in them that may be beneficial in alleviating the suffering of humanity caused by toxicity associated with synthetic anti-urease agents along with the development of resistance against them.

Nigella sativa L. (Black cumin) mainly contains thymoquinone and its derivatives as major ingredient; that are primarily attributed for its antioxidant, anti-inflammatory, anti-microbial and analgesic activities. These pharmacological activities are reported for their therapeutic effects on multi human systems (Hannan *et al*. 2021).

Ricinus communis L. (Castor plant) contains flavonoids, saponin glycosides, alkaloids and steroids. The rich presence of phyto constituents is responsible for the plant's anti-inflammatory, analgesic, antioxidant, immune-modulatory, anti-microbial activities (Jena and Gupta, 2012).

Ocimum sanctum L. (Tulsi) contains polyphenolic constituents. It is a rich source of voatile oils due to which it has potent antioxidant, anti-microbial, anti-inflammatory, analgesic, adaptogenic and immune-modulatory activities. Tulsi has broad spectrum therapeutic application and efficacy against various human organ pathologies (Cohen, 2014).

Curcuma longa L. (Turmeric) contains polyphenol, curcumin as its major constituent. Curcumin is responsible for its primary pharmacological activities including analgesic, anti-inflammatory, antioxidant anti-microbial and anti-cancer. Turmeric as a whole herb and its curcumin extract both have been explored and standardized by various researchers to be safe and effective for the treatment of different diseases (Soleimani *et al*. 2018).

2. MATERIALS AND METHODS

2.1. Chemicals

Folin–Ciocalteu, Na_2CO_3, gallic acid, phenol reagent, alkali reagent, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Butylated hydroxyanisole (BHA), were obtained from Sigma Chemical Co. All the other reagents were of analytical grade.

2.2. Plant material and extraction preparation

Nigella sativa, Ricinus communis, Ocimum sanctum, Curcuma longa were collected from the local market of Karachi, Pakistan in the month of January 2022. Each material (1 kg) was extracted with methanol for 15 days at room temperature. The resultant extract was concentra-

ted under vacuum using Buchi rotary Evaporator (Switzerland).

2.3. In vitro antioxidant activity

The antioxidant activity of *Nigella sativa (NS),Ricinus communis (RC), Ocimum sanctum (OS),Curcuma longa (CL)* were evaluated through DPPH method which is a free radical scavenging method (Srivasrtava *et al*., 2015). First the reaction mixture was prepared by adding 90 µL of DPPH solution and 10 µL of test sample solution respectively on microliter plate. It was then incubated at room temperature for 2hrs. After incubating, the absorbance of the samples was read at 517 nm against the blank using a spectrophotometer.The % inhibition of all extracts was measuredby formula:

%age inhibition = (OD of blank - OD of test sample) * 100/OD of blank

2.4. In vitro Urease inhibition activity

For the evaluation of anti- urease activity, Indophenols method was used which quantify the ammonia and the enzyme activity, and the resultant absorbance was measured at 625 nm using Microplate reader (Xiao *et al*., 2007). 40 ìL phosphate buffer with pH 8.2, 10 ìL of each extract and10 ìL of enzyme were incubated in 96 well plate at a temperature of 37 °C for 10 min respectively. After that phenol reagent and alkali reagent (40 ìL) were added respectively to each well. Thiourea was used as standard, and all experiments were carried out in triplicate manner. The percentage inhibition was calculated by the formula:

$100-(OD_{test\ Well} / OD_{control}) * 100$

2.5. Statistical analysis

Experiment performed in triplicate manner. Statistical differences betweenthe experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Mean values were considered statistically significant when $p < 0.05$.

3. RESULTS AND DISSCUSION

In this study, the antioxidant activity of the extracts *NS,RC, OS,CL* was assayed by using DPPH method. The results indicated that the free radical power of CL (29.6±0.62) was stronger than other extracts i.e, NS (39.5±0.49), RC (42.1±0.56) when compared to BHA (44.2±0.24). Less activity was shown by OS extract (65.7±0.15). The results were presented in Table 1.

Table 1. Antioxidant activity of *Nigella sativa, Ricinus communis, Ocimum sanctum, Curcuma longa* extract

S. No	Extracts	Concentration	Inhibition (%)
1	NS	1 mg	39.5± 0.49*
2	RC	1 mg	42.1±0.56*
3	OS	1 mg	65.7±0.15
4	CL	1 mg	29.6±0.62*
5	BHA	0.5mmol/ml	44.2±0.24

Values were expressed as mean ± S.E.M. Statistical significance was calculated by ANOVA followed by Tukey's post hoc test * $p< 0.05$; when compared to control.

From the beginning of the civilization the association of humans and plants and their co-evolution have been started. The presence of the plants on consi-

dered and signified as the essence of human remedies for many centuries (Li *et al*, 2015; Locatelli *et al*., 2003). Previous decades, witnessed the remarkable research in the field of natural medicines. The therapeutic and pharmacologically active compounds in the medicinal plant served as a source to develop a lead drug but it also provide a guide to develop new drug candidate (Sadeer *et al*., 2019).

Under the oxidative stress reactive oxygen species (ROS) are generated. They act as a secondary messenger in many physiological manifestations and cause deteriorating and pathogenic part in heart diseases, brain disorders, cancer, inflammatory diseases, eye infections and atherosclerosis. The main function of antioxidants in the body is to maintain the redox balance (Di Meo *et al*., 2016; Khan Kawal KH *et al*., 2021). Therefore, the ability of the natural antioxidant system can be enhanced using dietary antioxidants. The synthetic antioxidants like BHA (Butylated hydroxyanisole) and BHT (butylated hydroxytoluene) in the food produce several side effects that why there is a need to explore new antioxidants from the natural source having less side effect (Abbasi *et al*., 2020).The presence of Phenolic acid, flavonoids and Tannins in a substance is responsible for its antioxidant properties. It was reported that selected medicinal plants are rich in these compounds. The strong and significant activity shown by *Curcuma longa*extract might be related to the presence of these secondary metabolites.

For anti-urease activity, the four medicinal plants were exposed to Urease inhibition assay and the results were shown in Table 2. The highest anti urease activity was shown by CL extract (29.8±0.39) when compared to the standard Thiourea (22.4±0.29). Followed by OS (45.7±0.42) extract, whereas NS and RC extracts (55.8±0.13, 49.9±0.98) were moderately less active. On the bases of results CLextract showed significant anti-urease activity.

Table 2. Antiurease activity of *Nigella sativa, Ricinus communis, Ocimum sanctum, Curcuma longa* extract

S.No	Extracts	Inhibition (%)
1	NS	55.8±0.13
2	RC	49.9±0.98
3	OS	45.7±0.42*
4	CL	29.8±0.39*
5	Thiourea	22.4±0.29

Values were expressed as mean ± S.E.M. Statistical significance was calculated by ANOVA followed by Tukey's post hoc test * $p< 0.05$; when compared to control.

Urease is the enzyme that catalyzes the hydrolysis of urea to ammonia and carbamic acid (Mazzei *et al*., 2020). The pathogenicity associated to the urease which is one of the virulent factor include ammonia encephalopathy, urine stone formation, hepatic coma and pyelonephritis (Svane *et al*., 2020). Above all, Helicobacter pylori releases urease enzyme, which is responsible to counteract the acidic environment and increases the pH, this will result in hydrolysis of urea. It will be then colonized in the stomach and induces either peptic or duodenal ulcer or gastric cancer (Krajewska *et al*., 2009). There are many antibiotics available i.e, PPI, amoxicillin, and clarithromycin for the treatment of H. Pylori. According to different re-

searches, it was reported that more than 50% of the global population is infected by *H. pylori*. Subsequentlyby the inhibition of urease activity, the tested extracts (NS, RC, OS and CL) could terminate *H. pylori* infection, and it will play a significant role to produce promising therapy for the treatment of ulcer (Zahid *et al*., 2015). More research in this direction is needed to explore the potential of these medicinal plants against anti-urease activity.

4. CONCLUSION

The current study has offered a new approach to exploit anti-urease and antioxidant perspective of *Nigella sativa,Ricinus communis, Ocimum sanctum,Curcuma longa* for therapeutic perseverance in recent cure of several diseases.

Author's Contribution

Hina Zahid supervised the entire work, manuscript writing, Farah Saeed participated in literature survey, Mehreen Latif performed the experimental work.

The authors confirmed that there is no conflict of interest.

5. REFERENCES

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