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Phytochemical Analysis and Antioxidant Activity of Commiphora Wightii L. (Guggul)

Qureshi Ikram, Chahar Om Prakash, Kachhawa GM
Department of Biosciences
Shri JJT University
Chudela, Jhunjhunu
Rajasthan

ABSTRACT:
The present study focus on Phytochemical Analysis and Antioxidant Activity of Commiphora Wightii L. (Guggul).The Phytochemical Analysis, β-Carotene Bleaching Assay, Anti-lipid peroxidation assay, Metal chelating activity, DNA damage inhibition efficiency, Determination of phenolic compounds (HPLC analysis) of Commiphora Wightii L. Extract were determined. In this all method of Commiphora Wightii L. extract exhibit good scavenging activity. The IC_{50} Value= 934µg/ml. The zone of color retention was 15 mm in β-Carotene Bleaching Assay. IC_{50} values was found to be 627µg/ml. in Anti-lipid peroxidation assay. IC_{50} of the extract for chelating activity was 951 µg/ml. DNA protective efficiency of the extract was also studied using UV-photolysed H_2O_2 driven oxidative damage to pBR322. HPLC analysis identified some of the major phenolic compounds in the extracts, which might be responsible for the antioxidant. The present study concluded that the guggul plant have a very good antioxidant activity. The future it can be used for treatment of cancer.

Keywords: Commiphora Wightii, Antioxidant Activity, DNA, Anti-lipid peroxidation assay, β-Carotene Bleaching Assay.

1. INTRODUCTION

Medicinal plants and the human have very old relationship in nature. Medicinal plants are used for thousands of years and renowned for their effectiveness in cure of many diseases. These Medicinal plant products are very effective to improve the healing the allergies [1]. More than 80 % of the population throughout the world is directly and indirectly based on the traditional health care [2]. He also reported that 25% of modern medicines they come from, plant sources and many others from the modern pharmacopoeia are in fact synthetic analogues of drugs from the selected plant species [3]. Reported that use of medicinal plant increase the immune system, raising and renewing the body vitality and increasing the body resistance to infection. Many plants are directly used as medicines in our normal life. [4, 5]. Guggal (C. wightii) [6] (Arnott) Bhandari comb.) is a bushy shrub or small tree. Guggul plant is found in India (Rajasthan west part of Gujarat), and Pakistan (Baluchistan and Sindh). Guggul is an endemic species which is found in small part of the world [7]. Plant reproduction is occurring by sexually process and this may occur by sexual distribution: bisexual and female flowers and male flowers other stamminodes [8]. The maximum height of plant (bushy appearance at ground level branching tree) is 2-3 m. the heavy, shrubby and under the foliage of the ash-flakes, the color of bark on an uneven surface, the physical basis of the description [9]. The guggul modern therapeutic purposes is also targeted for rheumatoid arthritis, neurological diseases, hypercholesterolemia [10], leprosy, muscle, skin disorders, high blood pressure, and urine, and hypolipidemic and antioxidant [11]. The rubber the stickiness and platelets, and Gugulipid was the effective and cost-effective treatment is the Hyper-lipoproteinemia [12]. In new research, it was found that oleo-gum-resin effective against cardiovascular diseases and cancer [13]. The present study focused on phytochemical analysis and antioxidant activity of commiphora wightii (guggul) plant.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Mature and healthy leaves of C. wightii were collected from the Rajasthan (Jhunjhunu) during January and September 2012. Plant samples were collected for the various experiments (microbial activity and other biochemical test), methods are given as under.

Guggul (C. wightii) plant was collected from January and September, 2012 from different Guggul (C. wightii) growing areas of Rajasthan. Gum resin of C. wightii was collected from Shekhawati region particularly- Jhunjhunu the Kharkara, Gawari and cherani villages near JJT University, Rajasthan (India).
The leaves samples were removed use sharp knife and store in clean polythene bags. Every sample was used for the experiment. Sample was stored in freeze for further use.
These medicinal plants identify by botanist Dr. Hanuman Prasad JJT University, Rajasthan (India).

2.2 Extraction of Guggul Plant

For the experiments, fresh plant leaves of C. wightii were collected from field in polybages with ice packs. These fresh leaves were with tap water and than double distilled water for thrice. The leaves were dried and then grinded into powder form and mixed with distilled water (10 gm of extract in 100 ml of distilled water). The extract was kept under rotary evaporator which was used to dry and concerted, after that these samples were which is kept in air tight container and stored under lower temperature.

2.3 Antioxidant activity of guggul plant

2.3.1 Composition of Extract

The 100 gm extract of resin is mixed with ethyl acetate for 5 days. Then filter with Whatman no. 2 paper it is made evaporated using low pressure then undergo chemical test for flavonoids, steroids, alkaloid, terpenoids, etc

2.4 β-Carotene Bleaching Assay

The β- Carotene Bleaching assay was carried out [14]. In molten agar (4% solution in boiling water, 10 ml), Linoleic acid solution (2 mg/ml, solution in acetone, 10ml.) was added. It was pour into petri plate (diameter 8 cm, 20 ml per dish) and kept in dark and left stand to allow agar to set. Holes (4-mm diameter) was punched in agar, extract (1 mg) each in DMSO was transfer in the hole and petri plate was incubate at 45^0C for 4 hrs. After incubation, zone of colour retention around the hole was measured.

2.5 Anti-lipid peroxidation assay

According to [15] anti-Lipid peroxidation was carried out. Different concentration of plant extract was mix with 2.8 ml. of 10% goat liver homogenate and 0.1 ml of 50 mM $FeSO_4$ and incubated for 30 mins at 37^O C. Following this, 1 ml of extract was taken with 2ml of 10 % TCA- 0.68 % TBA in acetic acid (50%) follows by boil for 1 h at 100^O C and subsequent centrifuge at 10000 rpm for 5 min. The absorbance was recorded at 535 nm. ALP percentage was calculating the following formula:-

$$\% \text{ ALP } = \frac{\text{absorbance of fe2 induce peroxidation} - \text{absorbance of sample}}{\text{absorbance of fe2 induce peroxidation} - \text{absorbance of con trol}} \text{x } 100$$

2.6 Metal chelating activity

The chelating activity of the plant extract for ferrous ions was calculated according to Dinis [16]. The reaction mixture containing 0.5 ml of plant extract, 1.6 ml of deionized water, 0.05 ml of FeCl (2 mM) and 0.1 ml of ferrozine (5 mM) and it was incubated at 40^o C for 10 mins. The absorbance was measured at 562 nm.

The chelating activity was calculated as
% Chelating Activity = [(A Where A01 - A2) / A0] ×100

$$\% \text{ Chelating Activity } = \frac{(\text{A Where A01 } - \text{ A2})}{\text{A0}} \text{x } 100$$

2.7 DNA damage inhibition efficiency

Plant extract was used to check the DNA damage inhibition [17]. DNA damage inhibition by plant extract was tested by photolysing H_2O_2 by UV radiation in presence of pBR322 plasmid DNA[17]. 1 µl aliquots of pBR322 (200 µg/mL) were taken in three polyethylene microcentrifuge tubes. Following this, 50 µg of plant extract was separately added to two tubes and third tube was left untreated as the irradiated control (CR). Three percentage of H_2O_2(4 µl) was added to all the tubes and it wereplaced on the surface of a UV transilluminator (300 nm). Itwas irradiated for 10 mins at room temperature. After irradiation, the samples were analyzed by gel electrophoresis on a 1% agarose gel in TBE buffer (pH 8).

2.8 Determination of phenolic compounds (HPLC analysis)

Phenolic compound in the plant extract were analyzed using the phenolic standard for HPLC [18-21]. HPLC analysis was performed using a Waters 2487 HPLC system consist of dual y detector (5 µm, 4.6 mm × 150 mm). Gradient elution was performed at 35^0C with the solution A (50 Mm sodium phosphate in 10% methanol, pH 3.3) and solution B (70% methanol) in the following gradient elution program: 0-15 min – 100% of solution A; 15-45 min – 70% of solution A; 45-65 min-65 % of solution A; 65-70 min-60% of solution A; 70-95 min - 50% of solution A; 95-100 min-0% of solution A. The Flow rate was 1 ml/min was and injection volume 20 & L (of 10 mg/ml extract solution). Detaction was monitored at diverse wavelengths for various phenolic compounds, 250 nm for benzoic acid, isoflavones and most anthraquinones; 280 nm for some flavones, flavanones, catechins, theaflavins and some antrhaquinones; 320 nm for cinnamic acid, most flavones and chalcones; 370 nm for flavonols; 510 nm for anthocyanins.

3.Results and Discussion

3.1 Antioxidant and β-carotien assay

The antioxidant activity of plant extract was measured by the β-Carotene/linoleic acid model system (Fig. 1). The zone of color retention was 15 mm, which was close to the value of positive control ascorbic acid (17 mm) (Fig. 2). All the values were expressed as Mean±SD; n=3. Results: IC_{50} Value= 934µg/ml

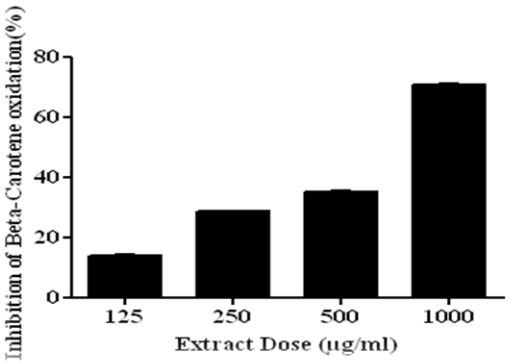

Fig 1: Beta-Carotene Bleaching assay

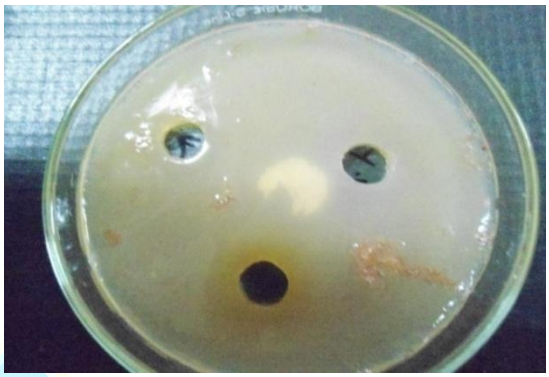

Fig. 2. β- Carotene Bleaching assay on petriplate

3.2 Antioxidant and Anti-lipid per oxidation assay

In Lipid peroxidation reaction, malondialdehyde (MDA) will release and it is react with thiobarbituric acid at low pH and a pink chromogen (TBA-MDA adduct) was formed. The best activity was seen at a high dose of 1000 µg/ml where 80% scavenging was observed and the IC_{50} values was found to be 627µg/ml (Fig. 3). Results were expressed in Mean ± SD; n=3

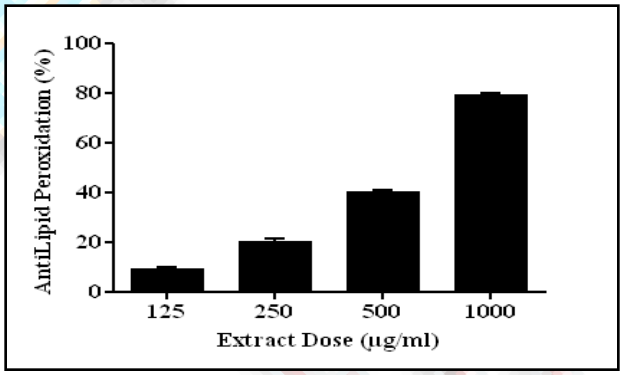

Fig 3: Antilipid peroxidation assay

3.3 Antioxidant and Metal chelating activity

The antioxidant from plant extract was interfere with the development of ferrous and ferrozine complex, signifying that it has chelating activity and captures ferrous ion before it react with ferrozine. IC_{50} of the extract for chelating activity was 951 µg/ml (Fig. 4). All the values were expressed as Mean±SD; n=3

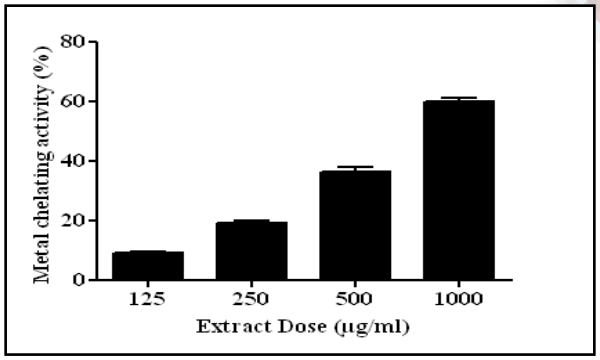

Fig 4: Metal chelating activity

3.4 DNA damage inhibition efficiency

A represented study in this pBR322 with / without guggul plant extract following UV-photolysis OF H_2O_2. The positive control pBR322 show a two band in agarose gel electrophoresis and negative control not show in any band (Fig. 5).

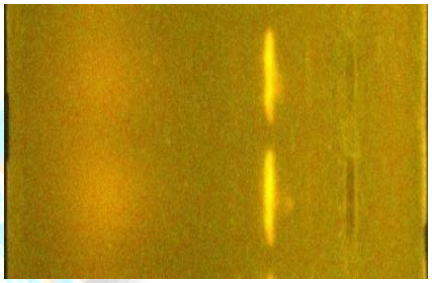

Fig 5: DNA damage inhibition efficiency

3.5 HPLC analysis of phenolic compounds

Plants containing the diversity of natural compounds, hence it is very difficult to identify them. Therefore, HPLC analysis was performed to identify the different phenolic compound present in the plant extract and it was compared with the reference retention times as reported earlier (Fig. 6). The major components in the plant extract were listed in table 1.

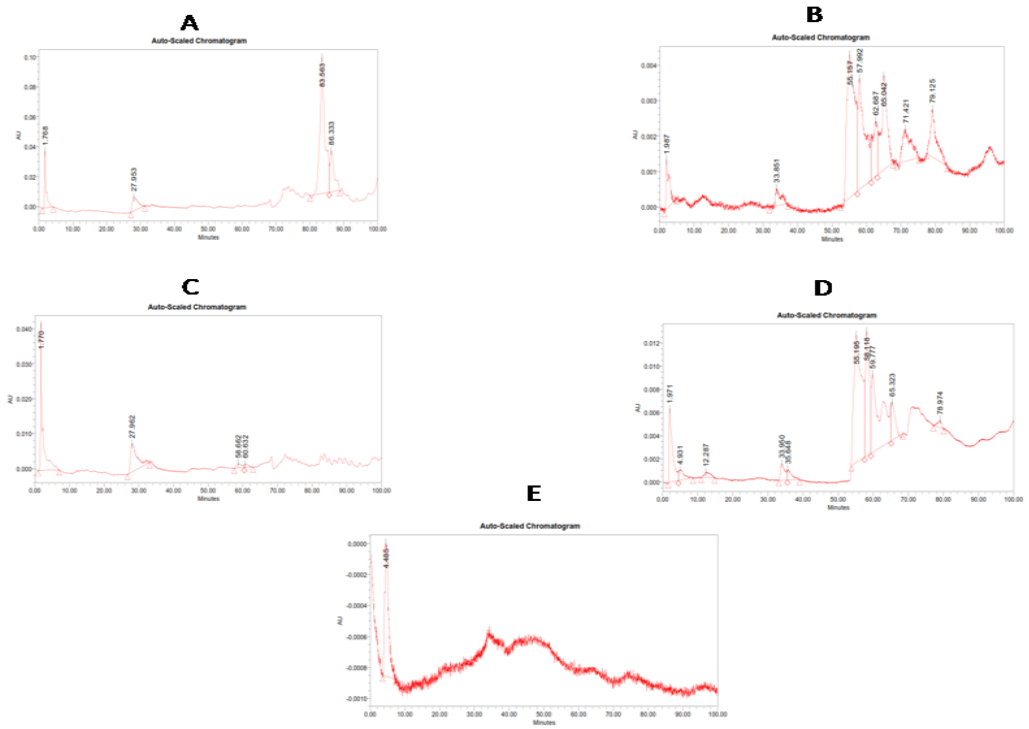

Fig 6: HPLC analysis

SAMPLE RT	REFERENCE RT	NANOMETER (nm)	COMPOUND NAME
27.953	25.6	250	Glycitein-7-o Glucoside (Glycitin)
83.563	83.4	250	Emodin
86.333	85.3	250	Rhein
27.962	26.1	280	(-) Catechingallate
35.648	35.2	320	Vitexin-2 –O rhamnoside
55.195	54.9	320	Luteolin-4-O glucoside
59.777	60.5	320	7,3,4- Trihydroxy flavones
33.851	34.0	370	Robinetin
55.157	55.6	370	Kaempferol-3-O-Glucoside (astragalin)
57.992	58.0	370	Kaempferol-3-O rutinoside

Table 1 Phenolic compounds identified in extract of plant extract by HPLC

DISCUSSION :

A medicinal plant is a great potential phytomedicine against the complex disease. According to WHO 80% world population is developed the medicine direct and indirect depend in health care product. The last few years have seen an increasing amount of knowledge about the important role of cancer diseases. These pathological and clinical backgrounds have promted to investigate noveland potent antioxidant compounds from Guggul plant extract which are ultimately of therapeutic use.

CONCLUSION:

The present study concluded that the guggul plant have a very good antioxidant activity. The future it can be used for treatment of cancer.

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REFERENCES:

1. Chandel, K.P.S., Shukla, G. and Sharma, N. (1996): Biodiversity in medicinal and aromatic plants in India: Conservation and Utilization. pp. 12.
2. Rao, M.R., Palada, M.C. and Becker, B.N. (2004): Medicinal and Aromatic plants in agroforestry systems.Agroforestry System.61: 107–122.
3. Kumar, S. and Shankar, V. (1982): Medicinal plants of the Indian desert: Commiphorawightii (Arrntt.) Bhand.Arid Environments. 5: 1-11.
4. Bhandari, M.M. (1964): Notes on Indian desert plants 4-new names and combinations. Bulletin of Botanical Survey of India.6: 327-328.
5. Bhandari, M.M. (1978): Flora of Indian desert, MPS reports, Jodhpur, pp. 89-90.
6. Sharma, M.L. (1994): Comparative chemical analysis of oleo gum resin of Commiphora wightii Arnott. Bhandari.Curr. Sci. 66(1):9 -10. Record from BIOSIS 1994:396732.
7. Atal, C.K., Gupta, O.P. and Afaq, S.H. (1975): Commiphora wightii: source of guggul in Indian system of medicine. Economic Botany. 29: 208-218.
8. Gupta, P., Shivanna, K.R. and Mohanram, H.Y. (1996): Apomixis and polyembryony in guggul plant; Commiphorawightii.Annals of Botany. 78: 67-72.
9. Kirtikar, K.R. and Basu, B.D. (1988): Indian Medicinal Plants. Vol. II. Internat. Book Distributors, Dehra Dun, India.
10. Nityananda, S. and Kapoor, N.K. (1971): Hypocholesterolemic effect of Commiphoramukul resin (Guggal).Indian J. Exp. Biol.9: 376-377.
11. Singh, R.B., Niaz, M.A. and Ghosh, S. (1994): Hypolipidemic and antioxidant effects of Commiphora mukul as an adjunct to dietary therapy in patients with hypercholesterolemia. Cardiovasc. Drugs Ther. 8: 659–664.
12. Satyavati, G.V., Gupta, A.K. and Tandon, N. (1987): Medicinal Plants of India.Vol 2.ICMR bull. New Delhi, India.
13. Xiao, D. and Singh, S.V. (2008): Guggulsterone-z, a constituent of Ayurvedic medicinal plant Commiphora mukul, inhibits angiogenesisin vitro and in vivo. Mol. Cancer Ther. 7: 171–180.
14. Loganathan Karthik, Gaurav Kumar, Kokati Venkata Bhaskara Rao. Antioxidant activity of newly discovered lineage of marine actinobacteria Asian Pacific Journal of Tropical Medicine (2013)325-332
15. Mandal TK, Chatterjee SN. Ultraviolet- and sunlight-induced lipid peroxidation in liposomal membrane. Radiat Res 1980; 83:290-302.
16. Dinis TC, Maderia VM, Almeida LM. Action of phenolic derivatives (acetomenophen,salicylate and 5- amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 315(1): 161-169.
17. Guha G, Rajkumar V, Mathew L, Kumar RA. The antioxidant and DNA protection potential of Indian tribal medicinal plants. Turk J Biol 2011; 35(2): 233-242.
18. Sakakibara H, Honda Y, Nakagawa S, Ashida H, Kanazawa K. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. J Agric Food Chem 2003; 51(3): 571-581.
19. Qureshi I and Chahar O P. medicinal use of endangered plant Commiphora wightii. Varioum Multi- Disciplinary e-Research Journal 2013; 4(I): 1-5
20. Chahar O P, Kharb P, Ali SF. Development of Protocol on Micropropagation in Ker (Capparis decidua (Forsk) Edgew) world Applied Science Journal. 2010; 10(6); 695-698
21. Sharma S, Chahar OP. Factors Affecting Exocellular Polysaccharide Production and growth by cyanobacterial strains. Variorum Multi-Diciplinary e- Research Journal, 2013; 4 (1); 1-9