



Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF GULKAND

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Abstract: Gulkand is a valuable medicinal formulation which has been valued for centuries in ayurvedic medicine. The aim of the present study was to evaluate *in vitro* antioxidant activities and to screen for phytochemical constituents of aqueous extract of gulkand. Phytochemical analysis revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, tanins, triterpenoids and saponins etc. *In-vitro* antioxidant activities were evaluated by DPPH free radical scavenging activity and reducing power which was found to be significant. In DPPH free radical scavenging assay, IC₅₀ values of extract and Ascorbic acid was found to be 39.82µg/ml and 525.6µg/ml respectively.

Keywords: Gulkand, phytochemical screening, antioxidant activity, DPPH- radical scavenging activity, reducing power.

INTRODUCTION

Oxidative stress occurs due to an imbalance between pro-oxidants and antioxidants and consequent excessive production of reactive oxygen species. Reactive oxygen species are biphasic, playing a role in normal physiological processes and are also implicated in a number of disease processes, whereby they mediate damage to cell structures, including lipids, membranes, proteins, and DNA.¹ A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital.² Antioxidants are substances that protect other chemicals of the body from damaging oxidation reactions by reacting with these free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation.³ An extensive range of antioxidant defences, both endogenous and exogenous, are present to protect cellular components from free radical induced damage. These can be divided into three main groups: antioxidant enzymes, chain breaking antioxidants, and transition metal binding proteins. Enzymatic antioxidants include antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase. The chain breaking antioxidants include lipid phase chain breaking antioxidants and aqueous phase chain breaking antioxidants. Lipid chain breaking antioxidants include Vitamin E, Tocopherols (α, β, γ, and δ). Aqueous phase chain breaking antioxidants include Vitamin C. Transition metal binding proteins (ferritin, transferrin, lactoferrin, and caeruloplasmin) act as a crucial component of the antioxidant defence system by sequestering iron and copper so that they are not available to drive the formation of the hydroxyl radical. At present most of the antioxidants are manufactured synthetically. They belong to the class of synthetic antioxidants. The main disadvantage with the synthetic antioxidants is the side effects when taken *in vivo*. Strict governmental rules regarding the safety of the food has necessitated the search for alternatives as food preservatives.⁴ So, now-a-days we are alternating for

medicinal herbs. Medicinal plants have been known to be most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases. A large number of spices and herbs have been evaluated by various researchers for their antioxidant effects^{5,6} achieve a favorable outcome. Large numbers of medicinal plants and dietary nutrients have been shown to possess gastro-protective activities such as *Curcuma longa* L *Embllica officinalis* G., *Magnifera indica* L. *Ocimum sanctum*, *Centella asiatica*, *Aloe barbedensis*, *Terminalia chebula*, *Momordica charantia* L, *Ginseng*, *Capsicum* etc.^{7,8,9,10,11,12}

Gulkand means rose petal jam is one of the most delicious Ayurvedic preparations which has been used for centuries for good health. *Rosa centifolia* (centi: 100 &folia: petals), are the commonly used species for preparing Gulkand. This plant belongs to Rosaceae family. The National Institute of Ayurvedic Medicine provides a list of the benefits obtained by consuming gulkand on a regular basis. It has been traditionally used as a cooling tonic to fight fatigue, lethargy, hyperacidity, dysmenorrhoea, fluid retention and heat-related conditions. It is also good for memory and used as good blood purifier (AYUSHVEDA).

MATERIALS AND METHODS

Collection of Material:

Gulkand is a sweet preserve of rose petals and is made primarily of rose petals. We have collected the marketed product of Gulkand, mfd. By Patanjali Yogpeeth, Ahmedabad, India.

Chemicals and Reagents

All chemicals were of highest purity (≥ 99.0%). Ascorbic acid, H₂SO₄, Methanol, Na₂CO₃, Phosphate buffer, K₃Fe(CN)₆, potassium ferric cyanide, 10% trichloroacetic acid (TCA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide, potassium dihydrogen phosphate, 0.1% ferric chloride, were procured from Hi-

media Pvt Ltd. All the absorbances were taken by UV-Visible spectrophotometer (UV 1800 – SHIMADZU).

Phytochemical screening^{13,14}

Phytochemical examinations were carried out for aqueous extract of gulkand as per the standard methods and results are depicted in Table no.1

INVITRO ANTIOXIDANT PARAMETERS

DPPH free radical scavenging assay^{15,16,17,18}

1ml of aqueous solution of extract of various concentrations of 10mg/ml, 20mg/ml, 50mg/ml were mixed with 3ml of methanolic solution of DPPH ($\mu\text{g/ml}$). Similarly 1ml of aqueous solution of ascorbic acid ($\mu\text{g/ml}$) was mixed with 3ml of DPPH solution. A mixture of 1ml of distilled water and 1ml of methanolic solution of DPPH ($\mu\text{g/ml}$) served as control. After mixing, all solutions were incubated in dark for 20 minutes and absorbance was measured at 517nm. The experiment were performed in triplicate and percentage scavenging activity was calculated as follows

$$\text{Scavenging Percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Reducing Power assay^{19,20,21}

1 ml of different concentrations of 10mg/ml, 20mg/ml, 50mg/ml of aqueous extract of *gulkand* were mixed with potassium ferricyanide (2.5 ml, 1%) 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken out to this 2.5 ml water 0.5 ml FeCl_3 (0.1%) were added absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power²²

RESULTS AND DISCUSSIONS

Preliminary Phytochemical Screening of Extract

The phytochemical screening of aqueous extract of gulkand showed positive results for carbohydrates, triterpinoids, steroids, alkaloids, glycosides, tanins, flavonoids, and saponins results are shown in the TableNo.1.

Table 1: Phytochemical Screening Of Aqueous Extracts Of Gulkand

Phyto Constituents	Water extract <i>Gulkand</i>
Detection of Alkaloids	
a. Mayer's test	+
b. Wagner's test	+
c. Dragandraft's test	+
Detection of Carbohydrates	
a. Molish test	+
b. Benedict's test	+
Test for Glycosides (Keller-killiani test)	+
Test for Flavonoids	
a. Lead acetate test	+
b. Alkaline reagent test	+
c. Ferric Chloride test	+

Test for saponins	
a. Foam test	+
b. Froath test	+
Test for Terpenes	-
a. Salkowski reaction	
Test for Tannin and Phenolic compounds	
a. 5% FeCl_3 solution	+
b. Lead acetate solution	+
c. Gelatin solution	+
Test for Triterpenoids & Steroids	+
Liebermann Burchard Test	

+: Positive indicates presence of phytochemical constituents

-: Negative indicates absence phytochemical constituents

In-Vitro Antioxidant Activity

Flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes.

Scavenging activities by the extract

The percentage inhibition of scavenging activities of the aqueous extract of the gulkand for DPPH was shown in Table 2 .The extract of gulkand showed appreciable free radical scavenging activities at the highest concentrations of 1000 $\mu\text{g/ml}$ on DPPH with percentage inhibition of 76.1%. In the present study, IC_{50} values of ascorbic acid and aqueous extract of gulkand for DPPH scavenging activity was found to be 39.82 $\mu\text{g/ml}$ and 525.6 $\mu\text{g/ml}$ respectively. (Fig 1).

The reducing power potentials of the extract

Figure 2 shows the reducing power potentials of the aqueous extract of the test in comparison with a standard ascorbic acid at 700 nm. The reducing capacity of the extract is significant indicator of antioxidant activity which was also found to be appreciable. Increasing absorbance indicates an increase in reductive ability. The results show that there was increase in reducing power with increase in concentration (Table 3).

Table no 2: Evaluation of aqueous extract of Gulkand for DPPH scavenging activity

S.No.	Concentration ($\mu\text{g/ml}$)	% scavenging activity of ascorbic acid	% scavenging activity of gulkand extract
1.	12.5	27.41 \pm 2.46	-
2.	25	45.19 \pm 1.61	-
3.	50	62.9 \pm 1.62	-
4.	100	80.64 \pm 2.46	23 \pm 2.46
5.	250	-	41.2 \pm 2.42
6	500	-	57.5 \pm 1.62
7	1000	-	76.1 \pm 1.61
8	IC_{50} ($\mu\text{g/ml}$)	39.82	525.6

Values are expressed as mean \pm SEM of triplicates.

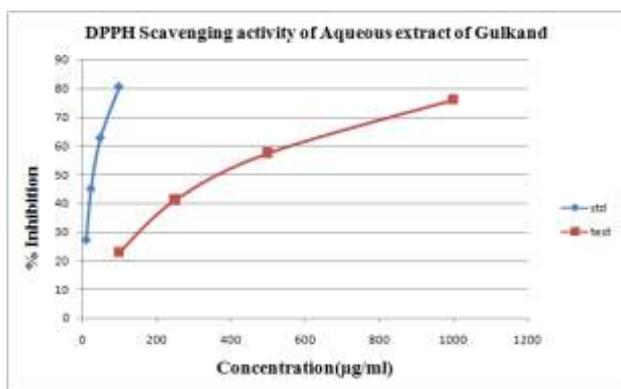


Fig No 1: Representing the DPPH scavenging activity of gulkand extract

Table 3: Evaluation of aqueous extract of Gulkand for reducing power

S.No.	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of Gulkand extract
1.	12.5	0.22	-
2.	25	0.48	-
3.	50	0.78	0.4
4.	100	1.19	0.5
5.	250	-	0.7
6.	500	-	0.9
7.	1000	-	1.2

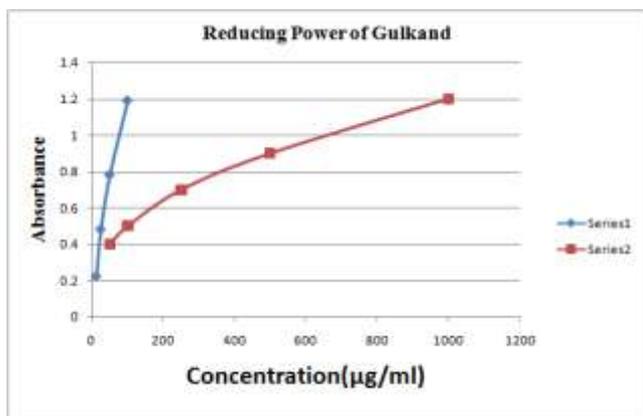


Fig No 2: Representing the Reducing Power of Gulkand

CONCLUSION

Rose Gulkand means rose petal preserve or rose petal jam. The National Institute of Ayurvedic Medicine provides a list of the benefits obtained by consuming Gulkand on a regular basis. Gulkand rich in calcium and antioxidant properties has been traditionally used as a cooling tonic

In the present study, the results of the two *in-vitro* antioxidant models i.e Reducing power and DPPH scavenging activities reveals that the aqueous extract of Gulkand had significant antioxidant activity. Further studies are required to confirm the exact mechanism underlying the antioxidant property of the extract and to identify the chemical constituents responsible for it.

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