

Biochemical Analysis and Mineral Composition of Methanolic Extract of *Astragalus Gummifer*

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ABSTRACT

Traditionally, gum obtained from *Astragalus gummifer* are consumed as analgesic agent. Current study focused on the proximate, phytochemical and elemental analysis of methanolic extract of *A. gummifer*. In proximate analysis different nutritional constituents like moisture, ash and total acidity in methanolic extract of *A. gummifer* was detected. In proximate screening highest ratio of ash ($35.1 \pm 2.0\%$) indicating a richest bio-resource of vitamins. While the values for moisture ($13.5 \pm 1.10\%$) and total acidity ($0.009 \pm 0.0001\%$) indicating its valuable properties. Phytochemical screening was implemented for the identification of different bioactive compounds i.e. tannins, flavonoids, gum and mucilage, phenols, saponins, protein & amino acid, alkaloids in methanolic extract of *A. gummifer*. The results indicated that flavonoids, phenolic compounds, proteins & amino acids and gum & mucilage were present in the extract while saponins and tannins were not found. Metallic screening was carried out for the quantification of different mineral elements i.e. Fe, Mn, Zn, Cr, Cu and Ni. The highest ratio was documented for Ni (8.69 ppm) followed by (4.73 ppm) for Cr. The lowest concentration was found for Mn measuring (5.30 ppm) while the values for Fe and Zn were recorded as (6.50 ppm and 6.04 ppm) respectively. The results obtained in this study clearly indicated that methanolic extract of *A. gummifer* is the rich source of secondary metabolites and certain mineral elements which support the use of this plant as stabilizing agent in the food, textile, leather, pharmaceutical, cosmetic and industries.

Introduction

Essential metals are valuable and show a unique role in the functional and structural integrity of the organization of living systems. Even though minerals encompass only 4-6% of the human body and do not play a part proficiently in fulfilling the energy necessities, however their significance can be recognized from their participation in a large number of physicochemical processes of supreme significance which occurs incessantly in living organisms [1,2]. Furthermore, minerals are generally classified as micro (zinc (Zn), manganese (Mn), magnesium (Mg), potassium (K), iron (Fe), copper (Cu), chromium (Cr) and cobalt (Co)) or macro (calcium (Ca), sodium (Na), chlorine (Cl) and phosphorus (P)) elements that generally depend on their requirements to the body and it

has been found that approximately more than 100 mg/dl of the macronutrients and less than 100 mg/dl of the micronutrients are essential on daily basis [3,4]. Insufficiencies of the mineral elements are the prime public health problem in lots of underdeveloped countries with women and infants [5]. Researchers have exposed that brutal cases of anemia most likely because of mineral deficiencies are a direct cause of child and maternal mortality. The herbal medicines are the best source of these fundamental minerals which can be consumed both for nutritional and therapeutic purposes [6].

Astragalus gummifer (Labill.) belongs to the family Fabaceae, is a naturally producing complex, synthesized from the shrubs of

the Astragalus plant and is commonly known as gond katira. Gum tragacanth is a native shrub found in arid regions of the eastern Mediterranean and South Western Asia [7]. Literature reported that Tragacanth gum could be used as laxative, antitussive, anti-diarrheic and as an aphrodisiac. Moreover, it is used as a binding agent in the formation of capsules, prescriptions and tablets, as an emulsifying agent in the production of creams, balms as an adhesive agent in pastes and as a thickener in the formulation of cosmetics, toothpastes, syrups, jellies, mayonnaise, salad dressings, sauces, liqueurs, candy and ice creams [8]. Literature further exposed that most of edible gum obtained from Astragalus genus has an extensive variety of applications in the pharmaceutical [9], cosmetic [10], and food industries [11-13], as a thickening emulsifying and gelling agent as they produce gel like viscous solution when added in water. In addition, pharmaceutical uses of gums as formulary mediator; the polysaccharides present in these gums also have potential biological actions [14,15]. Different polysaccharides contain various immunomodulatory properties depending on its structure including antioxidant [16], anticoagulant [17], hypocholesterolemic [18], antiviral [19], anti-inflammatory [20,21], keratinocytes, DNA repair [22] and antitumor [23-25] properties.

Furthermore, scientist performed an experiment on mice culture which is infested by Punta Toro Virus (PTV), Tragacanth in polysaccharides were introduced in the infected mice. The presence of these polysaccharides protected the majority of mice from mortality by activating the murin peritoneal macrophages, which proves that *A. gummifer* act as an immunomodulators and inhibit the multiple growth of cancer cells and viruses [26]. General survey highlighted that *A. gummifer* shows a potent allergenic effect and causing severe allergic responses. Further studies demonstrated that oral consumption or ingestion causes severe reactions. The immunogenicity of *A. gummifer* was established in mice that show cell- interceded immunity by performing swelling a test upon foot pad. Sanitization of the gum contended to a distinct decrease of the immune reaction [27]. No adverse effects were observed from hematological measurements and histological studies [28]. Moreover, plants gums are derivatives of different polysaccharides with a large number of biological activities and pharmaceutical applications. A small number of researches have focused on physicochemical properties of gums obtained from different plants especially that the gums which are available in herbal market are mixture of exudates obtained from different species. It is important to determine the physicochemical properties specifically metallic content of gum exudates obtained from different species of Astragalus genus to improve its biological application. Therefore, in current study we for the first time determined the elemental analysis of *A. gummifer*.

Materials and Methods

Sample Collection and Identification

The plant was selected from the local market of Peshawar, identified at the Shaheed Benazir Bhutto Women University Peshawar

(Khyber Pakhtoonkwha, Pakistan) and kept in polyethylene bags till analysis.

Nutritional Profile

The nutritional profile i.e. ash, moisture and total acidity of methanolic extract of *A. gummifer* was determined using the standard methods of Association of Official Analytical Chemist (AOAC) [29].

Phytochemical Screening

Phytochemical screening was performed for the identification of tannins, flavonoids, alkaloids, saponins, phenols, amino acids in the methanolic extract of *A. gummifer* by the standard methods of Association of Official Analytical Chemist [30].

Metallic Screening

Extract Preparation: *A. gummifer* extract was analyzed by Atomic absorption spectroscopy and dried in an oven at 70°C for 24 hours [31].

Digestion of Sample: *A. gummifer* (1g) was put in a porcelain crucible; the crucible was placed in a muffle furnace at 500°C for overnight. After this, the ash was then put in the 5mL flask by adding 20% HCL, followed by warming the solution through an acid necessary to dissolve the residue. After digestion the solution was then subjected to a filter paper washed by an acid for filtration and pour into a 50 mL flask. Finally, the filtered solution was then diluted by adding deionized water in it and shakes the solution well.

Results and Discussion

Nutritional Profile

The nutritional profile (ash, moisture and total acidity) of methanolic extract of *A. gummifer* in percent is given in (Figure 1). The calculated percentage of moisture content was (13.57 ± 1.10%) and the ash content was (92.3±3.38%). Similarly, the calculated value for total acidity was (0.009± 0.0001%). The proximate data of the *A. gummifer* provides valuable information about the prevalence and extent of macro elements which are mandatory for adequate ingestion of nutrients, normal physiological actions of human body and most frequently applicable for the elimination of illnesses. Nutritional profile of the methanolic extract of *A. gummifer* is given in Figure 1. In proximate analysis different compositions of nutritional constituents are studied. Highest ratio of ash (35.1 ± 2.0 %) shows that it is a richest source of minerals. While the moisture and total acidity shows valuable and reasonable association with their factors i.e. pressure, temperature and humidity. In the nutritional index the ash value is the total amount of inorganic material existing in the sample which are formed after the process combustion; hence illustrated the existence of greater amounts of organic components in the low ash values [32]. Furthermore, the presence of Ashes in the plant indicates the concentration of mineral elements in the plants which may affect the pharmacological activities of plant [33].

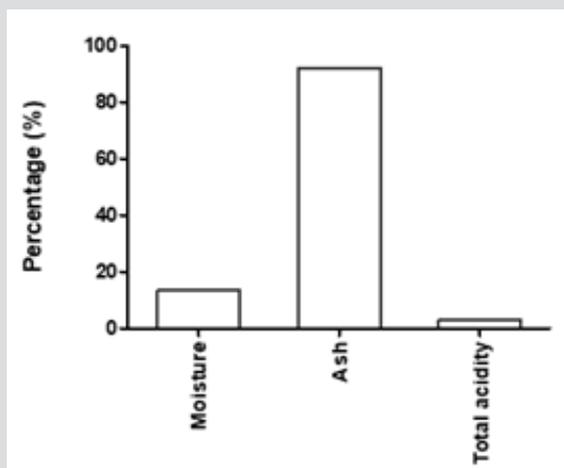


Figure 1: Nutritional profile of methanolic extract of *A. gummifer*.

In addition, moisture can be influenced by various environmental factors such as temperature, climate, humidity and harvest time as well as storage situation. Thus, it is important for nutritional scientist to quantify reliably the moisture contents [34]. The high moisture value indicates the amount of total solids and their short lifespan [35]. Low acidity value indicated that the plant do not have any aggressive effects and proves its safety and efficacy towards

Table 1: Qualitative analysis of the photochemical of methanolic extract of *A. gummifer*.

S#	Phytoconstituent	<i>Astragalus Gummifer</i>
1.	Alkaloid	+
2.	Saponins	-
3.	Phenolic compound	+
4.	Flavonoids	+
5.	Protein & amino acid	+
6.	Tannins	-
7.	Gum & mucilage	+

Similarly, flavonoids are a polyphenolic compound, naturally present in plants as secondary metabolites and have ability to perform important biological actions [39]. Flavonoids are also reported to inhibit NADPH oxidase activity, which are known to generate considerable amounts of O_2 radicals by phagocytes. Literature reported the in vitro vascular NADPH oxidase inhibition activity by a flavonoid derivative [40]. Flavonoids exhibit antioxidant efficacy via reinforcement of cellular antioxidants and are shown to act as intermediary antioxidants in the protection of lipophilic antioxidants (ubiquinol, vitamin E) and also endangered by hydrophilic antioxidants such as ascorbic acid [41]. The phenolic compounds are one of the major and most permeating groups of plants metabolites [42]. Phenols are known as anti-inflammatory agents, as natural antioxidants, effective against cardiac pathologies, potent anticancer agents and are also used as nutraceuticals [43]. They show biological activities like anti-aging, enhancement of endothelial capacity, anti-apoptosis as well as embarrassment of cell proliferation activities and angiogenesis

humans. The results achieved in the current study estimated that these plants are important for minerals consumption and energy requirements.

Phytochemical Screening

The phytochemicals (alkaloids, flavonoids, phenolic compound, protein & amino acid, saponines, tannins and gum & mucilage) were detected in methanolic extracts of *A. gummifer* as shown in Table 1. The results of *A. gummifer* indicated that flavonoids, phenolic compounds, proteins & amino acids and gum & mucilage were present in the extract while saponins and tannins were absent. In phytochemical analysis important secondary metabolites are studied as displayed in Table 1. Tannins are important, natural polyphenolic compound, having high molecular weights. Tannic acid is oxidized form of tannin that is accounted for scavenging $\bullet OH$ radical, it also inhibits 2-deoxyribose (peroxidative enzyme) and causes oxidative degradation influenced by $\bullet OH$ radicals, created from Fenton reagents [36]. In addition, it has capability to chelate trace metals like Fe (II) and cause the formation of complexes [37]. Furthermore, tannin also possess hydroxyl functional group which is responsible for confiscating heavy metal ions. Proanthocyanidine and flavan-3ol monomers are the types of condensed tannin that have been indicated for lowering plasma cholesterol levels, prevent LDL oxidation and activate eNOS to inhibit blood clot formation [38].

[44]. Proteins are important ingredients of cells; thus they are extensively important for human health. Literature revealed that protein-calorie malnutrition deficiency is a chief aspect accountable in dietary pathology [45]. The protein energy malnutrition (PEM) is considered as the main factor in development of various nutritional pathologies including kwashiorkor, marasmus or a combination of the two (marasmic-kwashiorkor). Thus, these results help in sorting the plants having high protein values and also encourage their uses in diet [46].

Metallic Content

The quantifiable mineral composition of *A. gummifer* shrubs was performed by atomic absorption spectrophotometer (AAS) for detection and estimation of metals including Fe, Mn, Zn, Cr, Cu and Ni as presented in Figure 2, which estimated that the evaluated elements were present in different fractions. The highest ratio was documented for Ni (84.69 ppm) followed by (41.73 ppm) of Cr. The lowest concentration was found for Mn measuring (5.30 ppm) while

the values for Fe and Zn were measured as (6.50 ppm and 6.04 ppm) respectively. Literature reported that Fe is a major constituent of hemoglobin and transferrin which is present in the blood cells and in plasma portions of the blood, respectively. Iron is also present in various portions of hemoglobin, myoglobin and cytochromes, and also serve as a succinate dehydrogenase as well as a co-factor for enzymes which take part in the production of neurotransmitters [47]. The deficiency of Fe in man causes weakening in development of brain, restive legs syndrome and organogenesis [48]. High intake of Fe in many body parts like tissues and organs as well as central nervous system subsequent in diseases like Parkinson's disease, haemosiderosis, Alzheimer's disease, type-1 neurodegeneration [49,50]. Besides, Fe in the body play an essential role as it transfers oxygen from lungs to the hemoglobin and various tissue cells [51]. Furthermore; its deficiency is the most inclusive nutritional discrepancy in person's resultant from minor intake of Fe in diet [52].

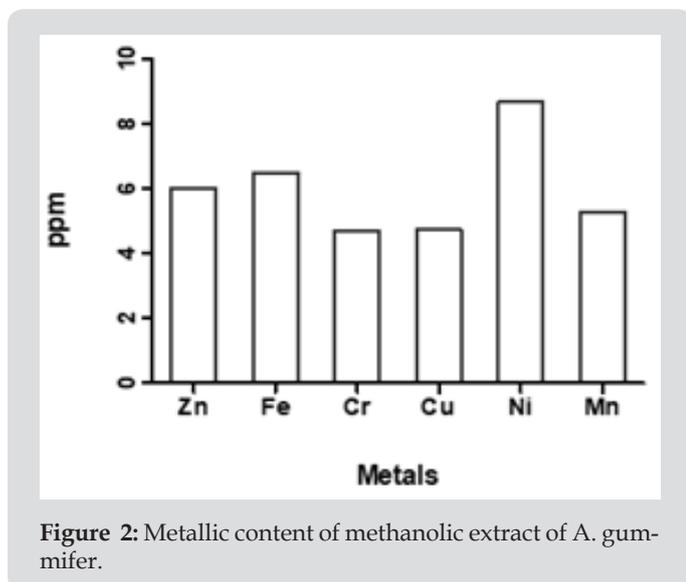


Figure 2: Metallic content of methanolic extract of *A. gummifer*.

According to FAO/WHO the permitted value for Cr suggested as 2 ppm for every individuals [53], which has closeness with the mean value of Cr in all the plants, which is ± 4 . Several studies reported that Cr is an imperious constituent of glucose tolerance factor (GTF) and effective component of carbohydrate metabolism, which increases the functions of insulin and endures tolerance of normal glucose level [54]. Deficiency of Cr causes certain diseases like atherosclerosis, hyperglycemia and cataracts. Human and animal study shows that Cr is helpful in regulation of RNA and also significant in the metabolism of lipids and proteins. Increase concentration of Cr causes toxicological effect in body tissues and also reduces the proper functioning of kidney, central nervous system, liver and blood and finally causes death of individuals [55]. Furthermore, the permitted level of Cu in diet is suggested as 900 mg for kids and adult persons. Cu is a significant redox-active constituent and a vital component of various organizations of enzyme comprising lactase, ceruloplasmin, cytochrome oxidase. The important role of Cu is to

assists the absorption and assimilation of Fe content in hemoglobin, and hence regulates the production of insulin while its deficit can cause disease like anemia [56]. Cu is also essential and active in the even functioning of hematologic and neurologic systems. But, additional intake of Cu in diet cause accumulation of Cu contents in liver which is effective in Cu poisoning deterioration in blood hematologic rate and Cu poisoning. It also unfavorably influences the regular activities of liver and most significantly causes jaundice owed to erythrocyte hemolysis [57].

Similarly, the permitted level for intake of Nickel (Ni) should be 0.006 ppm. Literature showed that Ni plays important role in the metabolic activities of nucleic acids, protection of the structure of membrane, regulator of prolactin and function as a basic component of plant enzymes such as hydrogenases and several ureases. Ni is usually found in the pancreas and reliefs in regulation of insulin whereas its deficiency causes certain disorders in liver [58]. Manganese (Mn) is an essential component of various biochemical processes and enzymes such as hydrolyses decarboxylases and transferases. Literature reported that Mn is effective in treatment of diabetes mellitus. Optimal level of Mn sustains the organization of normal bone and control the proper activities of central nervous system (CNS), while at low concentration they cause reproductive failure in both genders. Moreover, Mn toxicity results in severe influences on Central Nervous System and attitude [59, 60].

Conclusion

Current study highlights the significance of mineral composition, phytochemical constituents, and nutritional profile of *A. gummifer*, which confirmed that *A. gummifer* an edible gum and its constituents are significant and act as thickeners and adhesive agent in pharmaceuticals and industries. Furthermore, it can also be concluded that this medicinal plant is the valuable source of various pharmacologically active phytochemicals, nutritional constituents and certain mineral elements which help in regular physiological functions of the human body. Macro and micro nutrients perform a substantial role in the metabolic activities and formation of certain proteins like hemoprotein and hemoglobin of the body. As the world is become more advanced, the experimental data will provide new leads for researchers to exploit the valuable biological activities including their antimicrobial efficacy and antioxidant activity.

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