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Full Length Article

Comparative Evaluation of Phytochemical, Mineral and Vitamin Contents of Gemmomodified Extracts and Leaves of Two Indigenous Medicinal Plants

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Abstract

The good combination and significant concentration of phytochemicals and minerals is responsible for medicinal potential of plants. So this study has been planned to investigate and compare the phytochemicals, mineral composition and vitamin C contents of two indigenous medicinal plants *Datura stramonium* and *Nerium oleander.* Both gemmo modified extracts and leaves of plants were employed for analysis. Comparative analysis of gemmomodified extract and leaves of *N. oleander* showed that the amount of alkaloids, flavonoids, glycosides, tannic acid, steroids, triterpenoids and saponins was greater in leaves as compared to gemmomodified extracts. In case of *D. stramonium* amount of alkaloids and glycosides was greater in leaves, whereas the amount of flavonoids, tannic acid, steroids, triterpenoids and saponins was greater in gemmomodified extracts. The quantity of vitamin C, total phenolic and tannins was also greater in leaves as compared to gemmomodified extracts of both plants. Comparative study of minerals showed that the amount of Mn, Ca, Cu, Cr, Fe, and K was greater in leaves in case of *D. stramonium* whereas in case of *N. oleander* Cr, K and Zn were found in greater quantity in gemmomodified extract.

Key words: Gemmotherapy; Phytoconstituents; Micro minerals; Macro minerals; Ascorbic acid

Introduction

In the recent years there has been a gradual increase in the use of medicinal plant, because they are valuable natural resources and regarded as potentially safe drugs. These plants have been analyzed for biological, antimicrobial and hypoglycemic potential and also play a key role in the modern medicine (Hassawi and Kharma, 2006; Bhat *et al.,* 2009). The medicinal value of these plants depends upon chemical compound that produce a specific physiological action on human body (Edeoga *et al.*, 2005; Akinmoladun *et al*., 2007). About 60% of the total global population use medicinal plant for health care (Kumar *et al.*, 2004). Gemmotherapy uses buds of freshly growing plants or developing tissues during growth phase. These parts are rich in growth factors including auxins, gibberellins and phytohormones. The bioactive substances present in embryonic and growing tissues starts to disappear after maturation of plants. So the use of the young leafs, buds and rootlets make it possible to get a better medication compared to drugs that are prepared from the mature or whole plant (Hina *et al*., 2010). Gemmo-therapeutically formed medicines are safe, rich in nutrients and have positive effect for treatment and revival of human health (Jahan *et al.,* 2012). So the study of freshly growing parts (gemmomodified extract), along with native parts (leaves), of medicinal plants is very important.

Phytochemicals are the natural plant substances that play a key role in defense system against numerous diseases and stress condition (Hashmi *et al.,* 2013; Rahim *et al.,* 2013; Tupe *et al*, 2013). These phytochemicals are grouped into two main categories (Krishnaiah *et al.*, 2009) namely primary constituents, which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of flavonoids, alkaloids, saponins, tannins, tri-terpenoids and phenolic compounds (Edeoga *et al.*, 2005; Krishnaiah *et al*., 2007; Jign and Sumitra 2007; Kumar *et al.,* 2009). Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidal, antibacterial, antifungal, anti-constipative and antioxidant activities etc. (Abdeltawab *et al*., 2012). The plants thus find their medicinal value due to respective phytochemical constituents they contains.

Minerals are essentially required for tissue functioning in human beings. Minerals are substances that cannot be synthesize by living organisms and must be obtained from diet (Anjorin *et al*., 2010). Living organisms need minerals for osmotic adjustment, to activate enzymes and other organic molecules that enhance the growth and maintain life processes (Aslam *et al*., 2005; Anjorin *et al*., 2010).

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They are classified as micro and macro-minerals. Micro-minerals include Cr, Co, Zn, Cu, Fe, Mn and macro minerals include Ca, Mg, N, and K etc. Many important minerals that were found in medicinal plants are K, Na. Mg, Ca, Cu, Zn, Mn, Fe, Cr and Co.

Vitamin C also known as ascorbic acid is an important vitamin for human beings. It is very important antioxidant and protects the body from free radicals that can cause cancer. Vitamin C also facilitates transformation of cholesterol into the bile acid in the liver (Bender, 2003).

The present study was planned to determine and compare the phytochemicals, minerals and vitamin C contents of gemmomodified extract and leaves (native parts) of *D. stramonium* (datura) and *N. oleander* (oleander). Study of gemmomodified extract for these medicinal plants has been done for the first time. No such study and comparison has been done earlier. So the present study will be very helpful for the study of gemmomodified extract in future and this may be very helpful for the formulation of new and better drugs.

Materials and Methods

Sample Collection

Two plant species *D. stramonium* and *N. oleander* were collected from botanical garden "University of Agriculture, Faisalabad, Pakistan" for phytochemical screening, mineral composition and vitamin C estimation. Gemmomodified extract (extract of freshly growing parts) and leaves (native parts) of both plants were used for analysis.

Sample Preparation

The plant materials were washed thoroughly with water to remove dirt. Leaves of plants were dried in shade and ground to powder form whereas freshly growing leaves were used in the form of gemmomodified extract. These samples were stored in plastic bottles for further use.

Preparation of Gemmo-modified Extract

Gemmo-modified extracts of both plants were prepared by mercizating freshly growing plant material into a mixture of glycerin and methanol having a ratio of 1:2, respectively. The mixture was then allowed to stand for twenty one days in a cool, shaded environment, and shaken from time to time to facilitate maceration. It was then filtered under constant pressure. The resulting liquid known as stock solution was then evaporated in rotary to remove alcohol. This stock solution or extract (Gemmo-therapeutically treated plants) can be used within five years from the date it was prepared (Khursheed *et al.,* 2010).

Phytochemical Analysis

Alkaloids were qualitatively detected by dragendroff reagent (potassium bismuth iodide), Mayer's reagent (potassium mercuric iodide), Mayer's and Wagner's reagent (potassium iodide solution) (Harborne, 1973). Glycosides flavonoids were detected by Benedict's solutions (Adam *et al*. 1970), Fehling's solution 1 and 2 by SATS-OTTO procedure (Brain and Turner, 1975). Steroids and triterpenoids were detected by Liberman Barcharal reagent (Harborne, 1973). Anthraquinone was detected by magnesium acetate in methanol (Shibata *et al*., 1950). Tannic acid was detected by methods adopted by (Siddiqui and Ali, 1997). Quantitatively alkaloids, flavonoids, crude glycosides and steroids, and triterpenoids were extracted by using method of Brain and Turner (1975). Extraction of saponin was carried out by following the procedure described by Sharma *et al*. (1982), whereas method described by Hagerman and Klucher (1986) was followed for the extraction of tannic acid.

Determination of Total Polyphenols, Tannins and Vitamin-C

Total phenolic and tannins contents in gemmomodified extract and leaves of *D. stramonium* and *N. oleander* were determined spectrophotometerically (Okwu, 2005) by Folin-Ciocalteu method (Jahan *et al.,* 2011). Amount of Vitamin C was estimated by using titration method using 2, 6-dichlorophenol indophenol dye solution (Okwu, 2005).

Mineral Analysis

A known amount of plant material was demosturized by placing it in an oven at 105°C till constant weight was obtained and then this material was placed in covered crucible and ashed in furnace at 550°C for 5 h, then dissolved this ash in the concentrated nitric acid for determination of mineral contents.

Amount of Ca and Mg was determined by titration method using murexide and Eriochrome black T indicator respectively. Amount of Na and K was determined by flame photometer (Greenberg *et al.,* 1992). Concentration of Co, Cu, Cr, Fe, Mn and Zn was determined in gemmomodified extract and leaves of both the plant species with Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (1990).

Statistical Analysis

Each experiment was done for three concordant readings and data was expressed as Mean ± standard deviation. Means were tested with student t-test and significance accepted at $P \leq 0.05$ probability levels. T-test was applied individually on each experiment.

Results

Qualitative analysis of phytochemicals revealed that gemmomodified extract and leaves of *D. stramonium* and *N. oleander* are good source of alkaloids, flavonoids,

Table 1: Comparative study of phytochemicals of gemmomodified extracts and leaves of *Nerium oleander* and *Datura stramonium*

Phytochemicals	*N. oleander*		*D. stramonium*	
	Gemmo extract (%)	Leaves (%)	Gemmo extract (%)	Leaves (%)
Alkaloids	0.1±0.02[a]	0.3±0.05[b]	0.1±0.04[a]	0.5±0.02[b]
Glycosides	0.2±0.23[a]	0.3±0.23[b]	0.3±0.21[a]	0.4±0.3[b]
Flavonoids	1.6±1.12[a]	14.4±1.52[b]	18±1.32[a]	15.8±1.23[b]
Tannic Acid	4.5±1.42[a]	14.6±1.93[b]	8.5±2.11[a]	7.7±1.11[b]
Saponins	56.6±2.14[a]	71±3.21[b]	63.3±2.15[a]	62.6±2.34[b]
Steroids and triterpenoids	1.3±0.52[a]	7±1.27[b]	0	0

Data is expressed as Mean ± Standard Deviation. [a,b] Significantly different (P≤ 0.05) from each other

Table 2: Total polyphenolic, tannins and Vitamin C contents of gemmomodified extracts and leaves of *Nerium oleander* and *Datura stramonium*

Phytochmicals	*N. Oleander*		*D. Stramonium*	
	Gemmo extract (mg/g)	Leaves (mg/g)	Gemmo extract (mg/g)	Leaves (mg/g)
Tannins	120±1.23[a]	610±2.42[b]	420±1.87[a]	600±3.03[b]
Total polyphenol	110±1.42[a]	520±2.17[b]	350±2.01[a]	600±2.94[b]
Vitamin C	230±2.14[a]	330±1.73[b]	270±1.48[a]	430±1.75[b]

Data is expressed as Mean ± Standard Deviation. [a,b] Significantly different (P≤ 0.05) from each other

glycosides, tannic acid and saponin, whereas steroids and triterpenoids were only present in gemmomodified extract and leaves of *N. oleander* and was absent in gemmomodified extract and leaves of *D. stramonium.* Anthraquinone was absent in both plants. Quantitatively, *N. Oleander* possessed significantly (P ≤ 0.05) greater amount of alkaloids (0.3%), flavonoids (14.4%), glycosides (0.3%), tannic acid (14.6%), steroids and triterpenoids (7%) and saponins (71%) in leaves as compared to its gemmomodified extract. In case of *D. stramonium,* the amount of alkaloids (0.5%) and glycosides (0.4%) was significantly (P ≤ 0.05) greater in leaves, whereas the quantities of flavonoids (18%), tannic acid (8.5%), saponins (63.3%), steroids and triterpenoids were significantly (P≤0.05) higher in gemmomodified extract (Table1).

Comparative study of gemmomodified extract and leaves of *D. stramonium* revealed that the amount of total phenols, tannins and vitamin C was significantly (P ≤ 0.05) greater in leaves (610, 520 sand 330 mg/g, respectively) as compared to gemmomodified extract (120,110 and 230 mg/g, respectively). However, a comparative study of gemmomodified extract and leaves of *N. oleander* showed that the amount of total phenols, tannins and vitamin C was also significantly (P ≤ 0.05) greater in leaves (600, 600 and 430) as compared to gemmomodified extract (420, 350 and 270 mg/g, respectively) (Table 2).

Comparative study of minerals showed that the quantity of Mn, Ca, Cu, Cr, Fe, and K was significantly (P≤0.05) greater in leaves, whereas the amount of Mg, Na and Zn was significantly (P ≤ 0.05) higher in

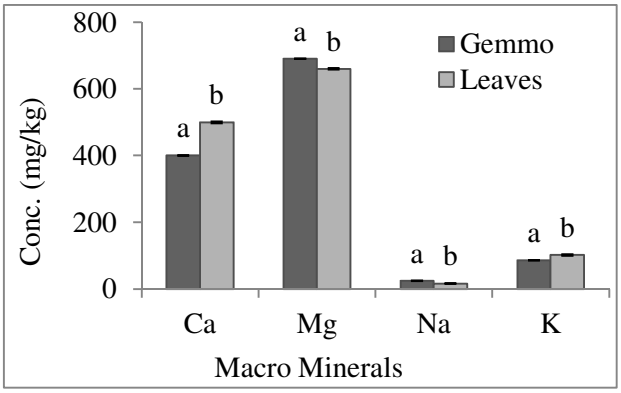

Fig. 1a: Comparative study of macro minerals of gemmomodified extracts and leaves of *Datura stramonium.* [a,b] significantly different (P≤ 0.05) from each other

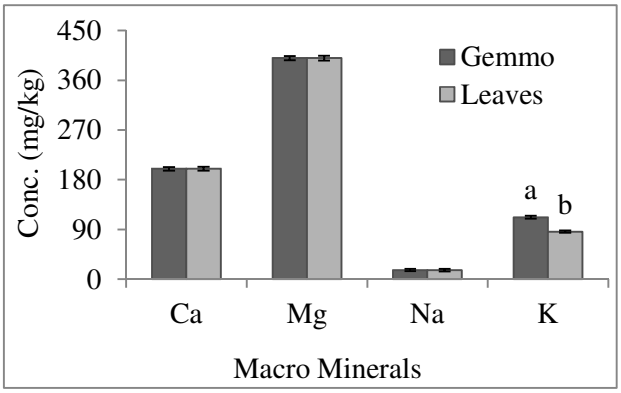

Fig. 1b: Comparative study of macro minerals of gemmomodified extracts and leaves of *Nerium oleander.* [a,b] significantly different (P≤ 0.05) from each other

gemmomodified extract and the difference in the amount of Co was non-significant in gemmomodified extract and leaves of *D. stramonium*. In case of *N. oleander* amount of Cr, K and Zn was significantly (P ≤ 0.05) greater in gemmomodified extract, whereas the quantity of Fe and Mn was significantly (P ≤ 0.05) higher in leaves and no significant difference was found between the amount of Co, Cu, Ca, Mg and Na in gemmomodified extract and leaves of *N. oleander* (Fig. 1, 2).

Discussion

Phytochemical analysis of *N. Oleander* (leaves) reveled that saponins were present in highest amount followed by tannic acid, flavonoids, steroids and triterpenoids, alkaloids and glycosides. In case of *D. stramonium* (gemmomodified extract) saponins were present in highest concentration followed by flavonoids and tannic acid. Highest saponins contents of plants are responsible for their anti-tumor and anti-mutagenic properties and can reduce the risk of human

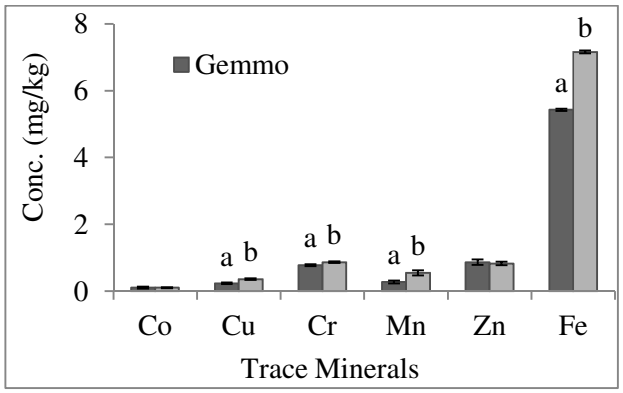

Fig. 2a: Comparative study of trace minerals of gemmomodified extracts and leaves of *Datura stramonium.* [a,b] significantly different (P≤ 0.05) from each other

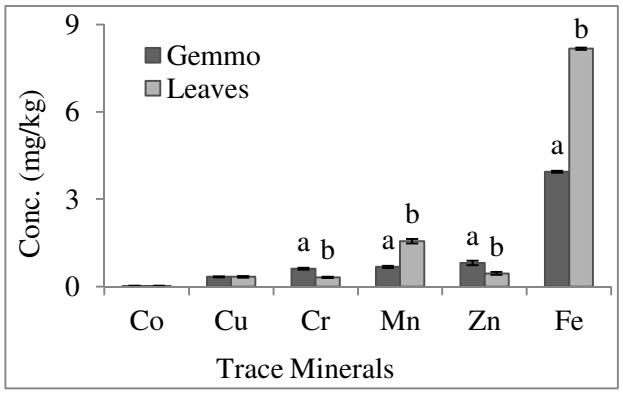

Fig. 2b: Comparative study of trace minerals of gemmomodified extracts and leaves of *Nerium oleander.* [a,b] significantly different (P≤ 0.05) from each other

cancer, by preventing the growth of cancer cells. Rao and Sung (1995) found that saponins aids in preventing colon cancer. Anti-hyperglycemic potential of saponins has also been reported (Nafiu *et al*., 2011). Tannic acid is a naturally occurring plant phenol present in fruits and vegetables and used as an additive in medicinal product for human. It is extensively used in the treatment of burns, diarrhea and as a local astringent (Delazar *et al*., 2003). The highest concentration of flavonoid in gemmomodified extract of *D. stramonium* may be attributed to the fact that gemmo parts (freshly growing parts) of medicinal plants contain many flavonoid contents that start to disappear after plant reaches a certain stage of development (Khursheed *et al*., 2010; Raziq *et al.,* 2012; Ayesha *et al*., 2013). These flavonoid contents are mainly responsible for the antioxidant activity of medicinal plants. The biological functions of flavonoids include protection against allergies, inflammation, ulcer and tumor (Okwu and Okwu, 2004). They are free radical scavengers, super antioxidants and potent water soluble thus preventing oxidative damage and have strong anti-cancer activity (Nafiu *et al*., 2011). Thus, the acclaimed anti-inflammatory uses of *D. stramonium* and *N. oleander* may be attributed to flavonoids.

Steroids and triterpenoids are anti-inflammatory, antimicrobial and analgesic agent (Singh, 2006) and play an important role in regulation of human hormones. Alkaloids are the most competent and therapeutically important plant substances. Pure alkaloids and their synthetic derivatives are used as an important medicinal agent because of their pain-relieving and anti-bacterial properties (Njoku and Akumefula, 2007). This may justify the use of *D. stramonium* and *N. oleander* in the treatment of pain, malaria and enteric fever in folk medicine. The cardiac glycosides therapeutically have the capability to enhance the power of the heart beat without any increase in the amount of oxygen required by the heart muscle. They can thus increase the efficiency of the heart without any strain to the organ (David, 1983).This has justified the use of *D. stramonium* and *N. oleander* in the treatment of heart diseases.

Greater concentration of vitamin C, total phenolic and tannin contents was found in leaves of both plants. This may be because as plants reaches to maturity light intensity increase the amount of ascorbic acid and the concentration of glucose, the precursor of ascorbic acid (Mozafar, 1994; Howard *et al.,* 2000). Ascorbic acid has an important role in preventing common degenerative conditions including heart diseases, cancer, cataracts and immune system functioning (Iqbal *et al*., 2004). Total phenolic contents generally increased with maturation, as reported in family Solanaceae (Howard *et al.,* 2000). Phenolic compounds have antimicrobial properties. Phenol and phenolic compounds have been extensively used in disinfections. Thus the reported antimicrobial properties of both plants may be attributed to phenolic compounds. Plants with tannins are used for healing of wounds, varicose ulcers and burns (Nafiu *et al*., 2011).

Various important macro and micro-minerals were present in both plants. The mineral profile of these plants was also studied by Oseni *et al*. (2011) and Hussain *et al*. (2011) who studied the seeds of *D. stramonium* and leaves, roots, stem and seeds of *N. Oleander* respectively. No previous work was found on the mineral profile of gemmomodified extracts of these two plants. These important macro and micro minerals play important role in body (Ficsor *et al*., 2013). Ca helps in bone formation and blood coagulation. Co promotes the formation of red blood cell and serves as a component of vitamin B-12. Cu is an important trace element for humans and animals. Cr assists in glucose metabolism and helps to regulate blood sugar level by potentiating insulin and serving as a component of glucose tolerance factor. The main function of Fe is in the transport of oxygen to the tissue (hemoglobin) and is also involved in the processes of cellular respiration (Sanjay *et al*., 2010).

Mg is also important in the appropriate utilization of vitamin B and E and function with other minerals such as Ca, Na and K in maintaining fluid and electrolyte balance (Nafiu *et al*., 2011). Mn plays an important role in a number of physiological processes, as a component of numerous enzymes and an activator of other enzymes (Nafiu *et al*., 2011). K plays an important role in the regulation of acid base balance in the cell, water retention and is essential for protein biosynthesis by ribosomes (Sanjay *et al*., 2010). Normal functioning of nervous system depends on Na. It acts with other electrolytes particularly with K in the intracellular space to regulate the osmotic pressure and maintain proper water balance within the body. It is also required for the proper absorption and transportation of other nutrients across cell membrane. Zn plays a key role in the proper utilization of Ca and Mg in cellular function as well as it helps to promote wound healing, and in optimizing the immune defense system (Nafiu *et al*., 2011).

To conclude, both plants are good source of phytochemicals, minerals and vitamin C. In case of *N. Oleander* most of the phytochemicals were in greater quantities in leaves whereas they were found in larger quantities in gemmomodified extract in case of *D. stramonium*. Total polyphenols, tannins and vitamin C contents were in larger amount in leaves of both plants. Minerals analysis revealed that the amount of Mn, Ca, Cu, Cr, Fe, and K was greater in leaves in case of *D. stramonium* whereas *N. oleander* exhibited greater quantities of Cr, K and Zn in gemmomodified extract. Good concentration of these phytochemicals and minerals makes these plants therapeutically very important.

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