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Physiochemical as well as microscopic identification of *Andrographis paniculata* and *Acacia arabica*

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ABSTRACT

According to World Health Organization more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. For the study these two plants was selected: Andrographispaniculata and Acacia Arabica respectively.

Physiochemical as well as microscopic identification is important to authenticate the crude drug. For these different types of study design like Determination of Ash value, Determination of Total Ash, Acid insoluble ash value, Water soluble ash value, Sulphated ash, Determination of Extractive value, Extraction of Plant for Phytochemical screening, and Extraction by cold maceration. Under Quantitative Microscopy study: Determination of leaf constants, Stomatal number, Stomatal index, Vein termination number & vein islet number and Veinlet termination number was studied.

Results of ash values for both plants showed higher in Andrographispaniculata5.50% than4.48%.in Acacia arabica. The results of extractive value of A. paniculata and A.arabica showed maximum extractive value in alcohol as compared to other polar and nonpolar solvents. Quantitative microscopical study of powdered leaves of Andrographispaniculata and Acacia arabica showed stomatal index 94 and 172 respectively. Similarly, stomatal index of A. paniculata was 50.8 and A. arabica was 25. Palisade ratio of A. paniculata in the range of 6-8 and A. arabica 5-6.

Ash values are indicative to some extent of care taken in collection and preparation of drug for market and of foreign matter content of natural drug. The object of ash ing is to remove all traces of organic material interfering in an analysis of inorganic elements. Quantitative Microscopy is important tool in the identification of the plant and the differentiation of the closely related species. The mentioned results indicate that selected plant was genuine.

Key words: *Quantitative Microscopy study, Stomatal index, Determination of Ash value, Determination of Total Ash*

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [2]. Cardiovascular diseases are the leading cause of death globally [1]. Together they resulted in 17.3 million deaths (31.5%) in 2013 up from 12.3 million (25.8%) in 1990.Cardiovascular disease, including stroke, is the leading cause of illness and death in the United States. There are an estimated 62 million people with cardiovascular disease and 50 million people with hypertension in this country [3]. In 2000, approximately 946,000 deaths were attributable to cardiovascular disease, accounting for 39 percent of all deaths in the United States [4]. Epidemiologic studies and randomized clinical trials have provided compelling evidence that coronary heart disease is largely preventable [5].

According to WHO, there are 146 million people worldwide with trachoma. Symptoms of bacterial eye infections include burning; irritation, tearing and usually a mucopurulent or purulent discharge. Eyelids may be stuck together, particularly in the mornings. Although bacterial eye infections are usually considered to be self-limiting [6], if left untreated they may develop into more serious, sight-threatening conditions.

Traditional medicine is defined as the sum of all the knowledge and practices, whether applicable or not, used in diagnosis, preventive and elimination of physical, mental and social imbalances and relying exclusively on particular experience and observation handed down from generation to generation, whether verbally or in writing. In another way, a traditional medicine may also be considered as a solid amalgamation of dynamic medical knowledge and ancestral experience.

Andrographispaniculata Nees belonging to family Acanthaceae, is another valuable, widely used medicinal herb. Whole plant is of medicinal value [7]. The plant is commonly known as “King of bitters”. Several formulations containing Kalmegh are available in the market. Plant is cultivated widely in India. Kalmegh contains bitter principle –Andrographolide, 14-deoxy Andrographolide, neoandrographolide, andropanoside, andrographiside and flavonoids. Andrographolide is commonly used as a marker compound to evaluate Kalmegh [8-9].

It is one of the herbs, which can be used to treat neoplasm as mentioned in ancient Ayurvedic [10]. Andrographispaniculata is reported as a cold property herb in Traditional Chinese medicine (TCM) and is used to get rid of body heat and to expel toxins. The plant is particularly known for its extremely bitter properties (often called King of Bitters) and is used traditionally as a remedy against common cold, dysentery, cardiovascular disease, fever, tonsillitis, diarrhoea, liver diseases, inflammation, herpes and so on [11]. The traditional uses and pharmacological aspects of *A. paniculata* have been well-documented in an extensive review recently [12]. A number of active principles are reported from the plant, which mainly include diterpenoid lactones, flavonoids, and polyphenols [13]. However, the prime constituent andrographolide has been mainly attributed for its therapeutic

properties. The diterpenoid lactone andrographolide, the principle compound found in A. paniculata, is mainly concentrated in leaves, and can be isolated from the crude plant extracts as crystalline solid [14].

Acacia is the most significant genus of family: Leguminosae, first described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of Acacia worldwide [15]. Acacia species—commonly known as Babool (or babul), Egyptian mimosa, Egyptian thorn, kikar, Indian gum, and red thorn—have long been used for the treatment of various ailments. Out of several species; Acacia Arabica is one of the species that has been effectively utilized in folk medicine [16]. It has been recognized worldwide as a multipurpose tree (National Academy of Sciences 1980). It is naturally widespread in the drier areas of Africa, from Senegal to Egypt and down to South Africa, and in Asia from Arabia eastward to India, Burma and Sri Lanka. The largest tracts are found in Sind [17]. It also serves as a source of polyphenols.

The role of these polyphenols to the plant itself is not well implicit, but for the human kind they can be of prime strategies. The photochemical contribute chemically to several groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes [18]. The bark, root, gum, leaves and flowers have found use for skin diseases, diarrhoea, dysentery, cough, diabetes, eczema, wound healing, burning sensation and as an astringent, demulcent, anti-asthmatic. The tender twinges are used as toothbrushes [19].

MATERIALS & METHOD

Determination of Ash value

The residue remaining after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.

The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. The ash remaining after complete ignition of the medicinal plant materials is determined by three different methods known as Total ash, Acid-insoluble ash and water soluble ash.

Determination of Total Ash

About 2 g of the powdered drug was weighed accurately and spreaded as a fine layer at the bottom in a tared silica crucible. The crucible was incinerated at a temperature to 500-600°C until it is white, indicating the absence of Carbon. The crucible was cooled and weighed. The entire procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated with reference to the weight of the air-dried drug using following formula:

$$\% \text{ Total Ash Value} = \frac{wt.of\ total\ ash}{wt.of\ crude\ drug\ taken} \times 100$$

Acid insoluble ash value

The ash obtained in the total ash was boiled with 25 ml of hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred to a tared silica crucible together with ash less filter paper and ignited at a temperature not exceeding 600˚C, cooled and weighed. The procedure was repeated till a constant weight was observed. This measure the amount of silica present, especially as sand and siliceous earth. The percentage of acid insoluble ash was calculated with reference to the air-dried drug using following formula:

$$\% \text{ Acid Insoluble Ash Value} = \frac{wt.of\ acid\ insoluble\ ash}{wt.of\ crude\ drug\ taken} \times 100$$

Water soluble ash value

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water. The ash obtained as described in the total ash, was boiled with 25 ml of hot water for 5 minutes and filtered on an ash less filter paper, washed with hot water. The insoluble ash was transferred to a tarred silica crucible and ignited at 600°C. The procedure was repeated to get a constant weight. The weight of the insoluble matter was subtracted from weight of the total ash. The difference in weight was considered for water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the air-dried drug using the following formula:

$$\% \text{ Water soluble ash value} = \frac{wt.of\ total\ ash - wt.of\ water\ insoluble\ ash}{wt.of\ crude\ drug\ taken} \times 100$$

Sulphated ash

A silica crucible was heated to redness for 10 minutes, allowed to cool in a desiccator and weighed. 1 to 2 g of the test drug substance accurately weighed in to the crucible was ignited gently at first, until the substance is thoroughly charred. The residue was cooled and moistened with 1 ml of sulphuric acid, heated gently until white fumes are no longer evolved and then ignited at 800 ± 25°C until all the black particles disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool and then a few drops of sulphuric acid was added and ignited as before. It was allowed to cool and then weighed to give the Sulphated ash content.

Determination of Extractive value

The term “extraction” is used, pharmaceutically to indicate “The process of separating the medicinally active portion of plant or animal tissues from the inactive or inert components by using selective solvent in standard extraction procedure”. The products, thus obtained, are relatively impure liquids, semisolids or powders intended for oral or external use. The mode of extraction selected greatly depends on the texture and water content of the plant material and in the type of substance to be isolated.

Alcohol soluble extractive value

Accurately weighed 5 gm coarse powdered leaves of *Androhraphispaniculata* and *Acacia arabica* were macerated with 100 ml of alcohol (90%v/v) in a stoppered flask for 24 h, shaking frequently during first 6 h, and it was allowed to stand for 18 h, then it was filtered rapidly through filter paper, precaution was taken against excessive loss of alcohol. 25 ml of alcoholic extract was evaporated to dryness in a tarred dish and dried at 105°C and weighed. The percentage of alcohol soluble extracts was calculated about the air-dried drug using following formula:

Alcohol soluble extractive value% = (B-A) x 4 x 100/W

Where, A-Empty weight of the dish (g)

B-Weight of dish + residue (g)

W-Weight of plant material taken (g)

Water soluble extractive value [20]

Accurately weighed 5 gm coarse powdered leaves of *Andrographispaniculata* and *Acacia arabica* were macerated with 100 ml of chloroform water in a stoppered flask for 24 h, shaking frequently during first 6 h. and was allowed to stand for 18 h, then it was filtered rapidly through filter paper, precaution was taken against excessive loss of solvent. 25 ml of aqueous extract was evaporated to dryness in a tarred dish and dried at 105°C and weighed. The percentage of water soluble extracts were calculated with reference to the air-dried drug.

Water soluble extractive value% = (B-A) x 4 x 100/W

Where, A-Empty weight of the dish (g)

B-Weight of dish + residue (g)

W-Weight of plant material taken (g)

Extraction of Plant for Phytochemical screening [21]

The dried leaves of *A. paniculata and A. arabica* were reduced to fine powder (40 size mesh) and around 500 gm of powder was subjected to extraction with Alcohol (70%) by continuous hot percolation method (Soxhlet extraction method). After effective extraction solvent was concentrated and drying using rota evaporator. The residue obtained after evaporation was weighed and percentage yield was calculated.

The above residue was subjected to successive washed with Benzene, Chloroform, Ethyl Acetate, Ethanol. (Each time before extracting with next solvent the extracted material (Residue) was dried at room temperature). After the effective extraction, solvent was concentrated and dried, and the residue obtained by each solvent was weighed.

Extraction by cold maceration

The powders of both plants were subjected for extraction by cold maceration with daily shaking for seven days at room temperature to obtain aqueous and alcoholic extracts. 1% chloroform was added to avoid growth of fungi. Glass container was covered with aluminium foil and kept aside with intermittent shaking. The liquid extract was separated by filtration and the filtrate was concentrated. The dried extract was collected and preserved in desiccator. Both the extracts were then used for pharmacological investigations.

QUANTITATIVE MICROSCOPY

Determination of leaf constants

A piece of leaf was cut in the middle portion and boiled in chloral hydrate solution or treated with chlorinated soda. Upper and lower epidermis were peeled out and mounted on glycerine on a glass slide. The slide was observed under microscope. The following measurements or determination were calculated using stage micrometer and camera Lucida for its stomatal structure, epidermal pattern, vein islet pattern, vein termination pattern and palisade ratio.

Stomatal number: It is the average number of stomata per square mm of the epidermis of the leaf.

Procedure: Middle part of the leaf was cleared by boiling with chloral hydrate solution or alternatively with chlorinated soda. Upper and lower epidermis were peeled out separately with the help of forceps & kept it on slide and mounted in glycerine water. Camera Lucida and drawing board were arranged for making the drawings to scale. With the help of micrometre, 1mm square was drawn. Cleared leaf was placed on the stage. Those cells were also included of which at least half of its area lied within the square. Number of stomata and epidermal cell which were present in the area of 1 sq.mm were counted. Procedure was repeated for ten different fields

Stomatal index

Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stomata being counted as one cell.

Stomatal index was calculated by using following equation.

$S.I. = S/E+S \times 100$

S.I = Stomatal index, S = No. of stomata per unit area,

E = No. of epidermal cells in the same unit area.

Procedure: Surface preparation was mounted on stage with camera Lucida as in stomatal number. Epidermis cell and stomata were traced on drawing board. Number of stomata was counted, also the number of epidermal cells in each field. The stomatal index was the calculated using the above formula.

Vein termination number & vein islet number

Veinlet termination number is defined as the number of veinlet terminations per square mm of the leaf surface, midway between midrib of the leaf and its margin. A vein-islet is the small area of green tissue surrounded by the vein-islets. The vein islet number is the average number of vein-islets per square mm of a leaf surface. It is determined by counting the no. of vein-islets in an area of 4 square mm of the central part of the leaf between the midrib and the margin.

Procedure: A piece of the leaf was cleared by boiling in choral hydrate solution for about thirty minutes. Camera Lucida and drawing board were arranged for making drawings to scale. Stage micrometer was placed on the microscope; a square of 1mm was drawn through the microscope. The paper was adjusted so that the square was seen in the eye piece, in the centre of the field. Cleared leaf was placed on the slide. The veins which are included within the square were drawn, completing the outlines of those islets which overlap two adjacent sides of the square. The numbers of vein islets were counted in the square millimeter. Where the islets were intersected by the sides of the square included those on two adjacent sides and excluded those islets on the other sides. The average number of vein islets was determined from the four adjoining squares, to get the values for one sq. mm.

Veinlet termination number

Veinlet termination number is defined as the number of veinlet termination per sq. mm of the leaf surface, midway between midrib of the leaf and its margin. A vein termination is the ultimate free termination of veinlet.

Procedure: Middle part of the leaf was cleared by boiling with chloral hydrate solution or alternatively with chlorinated soda. Upper and lower epidermis were peeled out separately with the help of forceps & kept it on slide and mounted in glycerine water. Camera Lucida and drawing board were arranged for making the drawings to scale. With the help of micrometer, 1mm square was drawn. Cleared leaf was placed on the stage.

The veins which are included within the square were drawn, completing the outlines of those islets which overlap two adjacent sides of the square. The numbers of vein termination numbers were counted in the square millimeter. Where the islets were intersected by the sides of the square included those on two adjacent sides and excluded those islets on the other sides. The average number of vein islets was determined from the four adjoining squares, to get the values for one sq. mm.

RESULT AND DISCUSSION

The results of physicochemical analysis of crude leave powders of *Andrographispaniculata and Acacia arabica* are shown in Table 2. The average values of various parameters such as total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and moisture content are expressed as percentage of air-dried material. Each determination was carried out three times and then average values are reported.

Ash Values

Ash values are indicative to some extent of care taken in collection and preparation of drug for market and of foreign matter content of natural drug. The object of ashing is to remove all traces of organic material interfering in an analysis of inorganic elements. Total ash, acid insoluble ash and water soluble ash of *Andrographispaniculata* and *Acacia arabica* were obtained by reported methods. Results of ash values for both the plants are given in 1. Leaves of *Andrographispaniculata* showed higher total ash value (5.50%) than that of *Acacia arabica* (4.48%).

Table-1: Ash values of leaves of *Andrographispaniculata* and *Acacia arabica*

Name of The Plant	Ash Values (%W/W)			
	Total Ash	Acid Insoluble Ash	Water Soluble Ash	Sulphated Ash
Andrographispaniculata	5.50	1.75	2.15	0.5
Acacia arabica	4.48	2.55	0.55	0.6

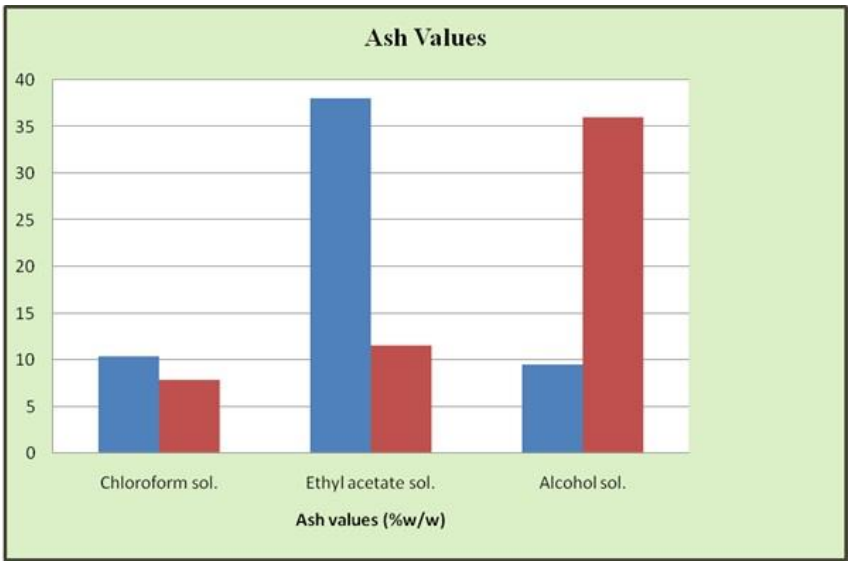

Figure-1: Graphical representation of Ash values of *Andrographis Paniculata* and *Acacia Arabica*

Extractive Values

It is employed for material to which as yet no suitable chemical or biological assays exist. Extractive values determine amount of active constituents present in given plant material in given solvent. Extracts were prepared with various solvents by maceration methods as described. Percentage of dry extract was calculated in terms of air-dried crude drug powder. The results of extractive value are given in 2. Leaves of *A. paniculata* and *A. arabica* both showed maximum extractive value in alcohol as compared to other polar and non-polar solvents. The characteristics of extracts for both plants like colour, odour and consistency were observed and noted in following tables 3 and 4. Extractive values can be compared for two plants and for different solvents with the help of graph figure 2.

Table 2: Extractive values of powdered leaves of *Andrographispaniculata* and *A. arabica*

Name of the Plant	Extractive Value			
	Chloroform soluble	**Ethyl acetate soluble**	**Alcohol soluble**	**Water soluble**
Andrographispaniculata	14.3	10.4	38.08	9.52
Acacia arabica	7.9	11.6	35.98	14.2

Table 3: Characteristics of *Andrographispaniculata leaves* Extract

***Andrographispaniculata* Extract**	**Colour**	**Odour**	**Consistency**
Chloroform	Greenish Brown	Characteristic	Sticky
Ethyl acetate	Green	Characteristic	Sticky
Ethanol	Dark green	Characteristic	Sticky
Water	Reddish Brown	None	Semisolid

Table 4: Characteristics of *Acacia arabica leaves* Extract

***Acacia arabica* Whole Plant Extract**	**Colour**	**Odour**	**Consistency**
Chloroform	Greenish Brown	Characteristic	Semisolid
Ethyl acetate	Greenish Brown	Characteristic	Sticky
Ethanol	Green	None	Solid
Water	Reddish Brown	None	Semisolid

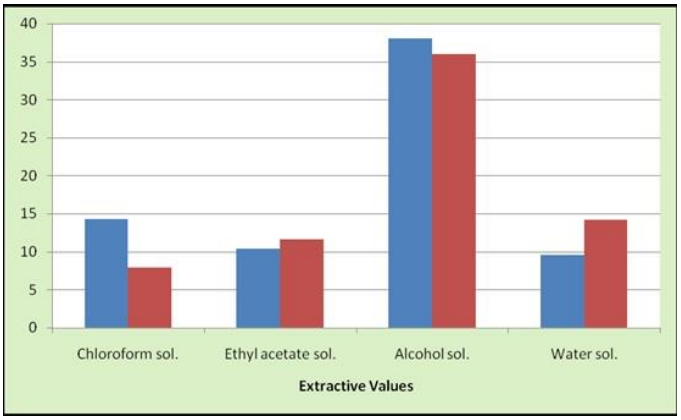

Figure-2: Extractive values of *Andrographispaniculata* and *A. arabica*

QUANTITATIVE MICROSCOPY

The important identifying characteristic of leaf constants like Stomatal Number, Stomatal Index, Vein-islet number, Vein termination number were found out and tabulated**.** These values are important tool in the identification of the plant and the differentiation of the closely related species.

Table 5: Quantitative microscopical parameters of powdered leaves of *A.paniculata* and *Acacia arabica.*

Parameter	***Andrographispaniculata***	***Acacia arabica***
Stomatal Number	94	172
Stomatal Index	50.8	25
Palisade Ratio	6-8	5-6
Crude Fiber content	18.24%	14.46%
Foreign Organic matter	Nil	Nil

CONCLUSION

Ash values are indicative to some extent of care taken in collection and preparation of drug for market and of foreign matter content of natural drug. The object of ashing is to remove all traces of organic material interfering in an analysis of inorganic elements. Quantitative Microscopy is important tool in the identification of the plant and the differentiation of the closely related species. The mentioned study indicates that selected plant was genuine.

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