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Pharmacognostical, physiochemical and phytochemical comparative study of the constituent of an antidiabetic polyherbal formulation

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Abstract

Multifactorial metabolic diseases develop several complications like hyperlipidemia, hepatic toxicity, immunodeficiency etc., Hence, instead of mono-drug therapy the management of the disease requires the combination of herbs. In the present study the investigation carried out on the pharmacognostical, phytochemical and physiochemical aspects of the leaf part of the plants. Polyherbal formulation of aqueous extracts of *Azadirachta indica*, *Camellia sinensis* and ethanol extract *asparagus racemosus* F1 (N: G: S=2:2:1) 200 mg/kg2:2:1, named F1, (N:G:S=2:1:2) 200 mg/kg2:1:2, named F2, (N:G:S=2:1:1) 200 mg/kg2:1:1, named F3. These Plants are boon to us from the nature as it claims its potentiality as a wide range of herb. The comparative Morphological studies of leaves showed the presence of various diagnostic characters. Ash value, extractive value and moisture content were determined for quality standard of drugs. During the course of the experimental work the leave part showed the presence of various phytoconstituents like flavonoids, tannins, protein, sterol etc.
Keywords: Phytochemical, Pharmacognostical, Physiochemical, Extractive value, Moisture content.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein, and lipid metabolism and by complications like retinopathy, microangiopathy, and nephropathy. According to WHO, diabetes mellitus will be the single largest non-communicable disease worldwide by the year 2025 with the largest diabetic population in India.[1] Current pharmaco-therapeutics/medicines are not able to reverse hyperglycemia completely, have limited tolerability, and induce side effects.[2]

Neem (*Azadirachta indica*., Family: Meliaceae, Subfamily: Meliodeae, Order: Meliales is an evergreen tree native to the Indian subcontinent. It also grows widely in several other countries of Asia, Australia, Africa and Central and South America[3]. More recent studies have focused on animals, including one report which indicates that neem's hypoglycemic effect is comparable to the prescription drug glibenclamide and noted that it may be beneficial in preventing or delaying the onset of disease[4].

Camellia sinensis is commonly known as tea. Tea is the most consumed drink in the world after water. Green tea is a 'non-fermented' tea and contains more catechins than black tea or oolong tea. Catechins are *in vitro* and *in vivo* strong antioxidants, there is also epidemiological evidence that drinking green tea (but not black tea or oolong tea) may help prevent diabetes[5].

Asparagus racemosus, traditionally known as shatavari means "who possesses a hundred husbands or acceptable to many". Aspargaceae is an ayurvedic plant with medical importance of tropical and subtropical India. Its medical usage has been reported in Indian & British Pharmacopeias and in traditional system of medicine Ethanol extract of A. racemosus causes lowering the blood sugar level[6].

2. Materials and Methods

2.1 Plant material

Leaves of *Azadirachta indica*, *Camellia sinensis* and *asparagus racemosus were* collected in the month of November 2011 from botanical garden of V.B.S.P University campus, Jaunpur, Uttarpradesh India. The plant was authenticated by Dr. A. K. Singh Head of the Department of Botany, T. D. P. G. College, Jaunpur, Uttarpradesh The leaves were cleaned and dried under the shade to avoid degradation of volatile oil.

2.2 Preparation of plant extract for Phytochemical Screening and Antidiabetic Studies

The *Azadirachta indica*, *Camellia sinensis* and *asparagus racemosus* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Azadirachta indica*, *Camellia sinensis* leaves was packed in a Soxhlet apparatus and extracted with water. Hundred grams of powdered *asparagus racemosus* leaves was packed in a Soxhlet apparatus and extracted with ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents.

2.3 Identification, evaluation and standardization

Identification of herb is based on macroscopical and microscopical features. Macroscopical feature involves odour, taste, color, size shape and special feature of plant and microscopically involves leaf content, trichome, stomata etc. Certain microscopical features and chemical test comes under evaluation and standardization of herbal drug. Evaluation of drugs means. Confirmation of its identity and determination of its quality and purity and detection of adulteration.[7]

Standardization expression is used to describe all measures which are taken during the manufacturing process and quality control leading to a reproducible quality. It's also involving the study from birth of plant to its clinical application. It's also include the herbal drugs preparation to a define content of a constituent or a group of substance with known therapeutic activity respectively by addition of excipients or by mixing herbal drugs preparation. In other words it's ensuring that every packet of medicine has correct ingredient in correct amount and will induce intended therapeutic effect.

Fig. 1- ***Aspergillus racemosus***

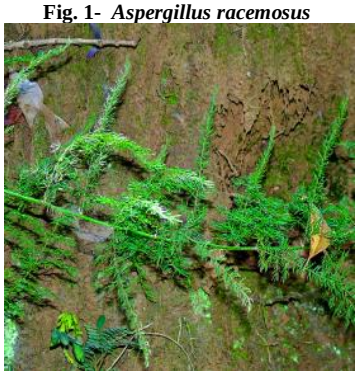

Fig. 2- ***Camellia sinensis***

Fig. 3 - ***Azardirachta indica***

2.4 Phytochemical screenings of herbal drugs

Extract were subjected for Phytochemical screenings to detect the presence phytochemical constituent viz. alkaloids, carbohydrates and reducing sugar, steroids, proteins, tannins, phenolic compounds, flavonoids, gums and mucilage, glycosides by using standard test .[8,9,10,11]

2.5 Evaluation Parameters of herbal drugs

2.5.1 Macroscopic evaluation

In this methods, description, general condition of the drug size, shape outer surface inner surface are referred. A sensory or organoleptic character describes colour, odour taste, consistency.[12]

2.5.2 Microscopic Evalution

The inner pseudoparenchyma cells are oval or rounded,the contain fixed oil & protein the whole tissue is devoid of cellulose and lignin.various parameter includes in microscopy.[13]

A. Leaf content
B. Trichome
C. Stomata

2.5.3 Physical E`valuation

A. Determination of foreign matter

It is the matter present in the drug Its presence may be due to faulty collection of crude drug or due to deliberate mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated. Drugs should be free from moulds insects, animal, faecal matter and other contamination such as earth stones and extraneous matters.

Percentage of foreign organic matter = n × W × 94,100 × 100 / S × M × P

Where: n= number of chart particles in 25 fields.
S= number of spores in the same 25 field.
W= weight in mg of lycopodium taken.
M= weight in mg of the sample (calculation on the sample dried at 105.c
P= number of characteristics particles per mg of the pure foreign matter.
94,000= number of spores per mg of lycopodium. [1-2]

B. Determination of total ash

The residue remaining after incineration is the ash content of drugs, which simply represents inorganic salts, naturally occurring in drugs or adhering added to it as form adulteration[14].

Two types ash determine-
(i) Acid insoluble ash value.
(ii) Determination of water soluble ash.

C. Determination of extractive value

(i) Determination of alcohol soluble extractive
(ii) Determination of water soluble extractive.

D. Determination of moisture content.-

Weighed 10 gm of drug and taken in a taken evaporating dish. Then it is dried 105°C for 3 hours and again weighed. Drying and weighing was continued at one hour interval until difference two successive weighing corresponds to not more than 0.25 percent. The reading is taken after a constant weight is reached and the moisture content is determined.[15]

E. Determination of pH

The pH value of an aqueous liquid may be defined as the common-logarithm of the hydrogen ion concentration expressed in grams. Potentiometrically pH value determine by a glass electrode and a suitable pH meter.[9]

F. Solubility

The presence of adulterant in a drug could be indicated by solubility studies identify by various solvents.[10]

i. Alcohol

5 gm of powdered material along with 100 ml of alcohol are shaken well occasionally for the first 6 hours and kept undisturbed for 18 hours. The liquefied extract thus obtained was concentrated in a vacuum oven and the percentage was calculated with the weight of the drug powder taken.

ii. Water

The procedure adopted for solubility percentage of alcohol is used with chloroform water instead of alcohol to get the water solubility.

G. Refractive index

When a ray passes from threw a one medium to another of different density, it is bent from original path. Thus, the ratio of velocity of light in vacuum to its velocity in a substance is termed as refractive index of the second medium. Depending upon purity, it's constant for a liquid and can be consider as one of its standardization. Refractive index of a compound varies with the wave length of the incident light, temperature and pressure.

H-Swelling Index

Specified quantity of plant material (5 g) concerned previously reduced to the required fineness and accurately weighed taken into 25 mL glass stopped measuring cylinder. 25mL of water was added and the mixture was shaken thoroughly every 10 min for 1 h. It was allowed to stand for 3 hrs at room temperature. The mean value of the individual determinations was calculated related to 1g of plant material.

I-Foaming Index

About 3 g of plant material was reduced to a coarse powder, weighed accurately and transferred at moderate boiling for 30 min. Cooled and filtered into 100 mL volumetric flask. The decoction was poured into 10 Ml flask and adjusted the volume of liquid in each tube with water to 10 mL. Stoppered the tubes and was shaken in a lengthwise motion for 15 sec.; two shakes per second. Allowed to stand for 15 min and the height of foam were measured.

3. Results

Table-1: Comparatively Moisture content (%w/w), Ash value (%w/w) and Swelling index of *Azadirachta indica, Camellia sinensis and Asparagus racemosus*

S. No.	Particular	Moister content (%w/w)	Total ash	Acid insoluble ash	Water soluble ash	Swelling index (in ml)
1	*Camellia sinensis*	3.3±0.14	0.3±0.05	0.1±0.04	0.06±0.04	4.65±0.05
2	*Asparagus racemosus*	2.9±0.23	4.3 ±0.05	0.5 ±0.13	0.8 ±0.11	3.20 ±0.45
3	*Azadirachta indica* (leaves)	3.4±0.44	5.2 ±0.15	0.6 ±0.07	0.4 ±0.21	3.40 ±0.32
All values are Mean (n) ± SD, n=3 and SD= Standard Deviation.						

Table-2: Comparatively Qualitative phytochemical screening of *Azadirachta indica, Camellia sinensis and Asparagus racemosus extracts*

S. No.	Test	Extracts					
		Camellia sinensis		*Asparagus racemosus*		*Azadirachta indica*	
		Water	Alcohol	Water	Alcohol	Water	Alcohol
1	Alkaloid	-	-	-	-	-	-
2	Carbohydrate	+	+	-	+	+	-
3	Glycoside	+	+	+	+	-	-
4	Phenolic Compound	+	+	+	+	+	+
5	Flavonoid	+	+	+	+	-	-
6	Volatile oil	+	-	-	-	+	+
7	Tannins	-	+	+	+	+	-

+= Presence, - = Absence

Table-3: Comparatively Morphological characters of *Azadirachta indica, Camellia sinensis and Asparagus racemosus.*

Parameters	*Camellia sinensis*		*Asparagus racemosus*		*Azadirachta indica*	
	Standard	Observed	Standard	Observed	Standard	Observed
Color	Green	Green	Cream	Cream	Yellowish green	Yellowish green
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Indistinct	Indistinct
Taste	Slightly bitter	Slightly bitter	Sweet	Sweet	Bitter	Bitter
Size	15-25cm long 0.1cm thick	15-25cm long 0.1cm thick	10-30cm long 0.1-0.5cm thick	8-10cm long 0.1-0.4cm thick	15-25cm long 0.1cm thick	10-22cm long 0.1cm thick

Table-4: Comparatively Extractive values (%w/w), Foreign matter content (%w/w) and Foaming index of *Azadirachta indica, Camellia sinensis and Asparagus racemosus.*

S. No.	Particular	Alcohol (%w/w)		Water (%w/w)		Foreign matter		Foaming index
		Observed	Std.	observed	Std.	Observed (%)	Standard (%)	
1	*Camellia sinensis*	12.23±1.7	<10	30±2.4	<35	1.8 ±0.13	< 2.0	< 100
2	*Asparagus racemosus*	14.8 ±1.4	<10	36±1.2	<45	0.8 ±0.14	< 1.0	< 100
3	*Azadirachta indica* (leaves)	15.2 ±0.11	<13	22.8 ±0.41	<19	1.02±0.16	<2.0	< 100
All values are Mean (n) ± SD, n=3 and SD= Standard Deviation.								

4. Discussion

Standardization of *Camellia sinensis, Asparagus racemosus* and *Azadirachta indica* (leaves) was preferred done by WHO guideline. The result obtained found under specified limits.

Morphological study is an important part for the identification of any drug. Morphological study showed that the drugs Camellia sinensis, *Asparagus racemosus* and *Azadirachta indica* (leaves) are having the characteristics as per the standards given in official compendia. Foreign matter is the material consisting of any organ other than those named in the identification and description, matter not coming from the source plant, molds, insects or other animal contamination. The results showed the content of foreign matter is 1.8 ±0.13, 0.8 ±0.14 and 1.02±0.16 respectively present in the drugs.

Moisture content used to determine the presence of volatile matter (i.e. water drying off from drug). The moisture content is 3.3±0.14, 2.9±0.23 and 3.4±0.44 respectively maximum moisture content.

The total ash value, show the presence inorganic matters in drug. Total ash (0.3±0.05, 4.3 ±0.05 and 5.2 ±0.15 respectively), acid insoluble ash (0.1±0.04, 0.5 ±0.13 and 0.6 ±0.07 respectively) and water soluble ash (0.06±0.04, 0.8 ±0.11 and 0.4 ±0.21 respectively) was

found in the drugs.

Foaming index was found in less than 100 in Camellia sinensis, *Asparagus racemosus* and *Azadirachta indica* (leaves) thus indicating absence of saponins. No Swelling was found to be 4.65±0.05, 3.20 ±0.45 and 3.40 ±0.32 respectively. It showed that polar constituents present in more quantity than non-polar constituents in the drugs.

The extractive value is used to indicate miscibility and presence of constituents in particular solvents, it is performed in solvents like alcohol and water, thus indication presence of more polar compound on moving on polarity scale.

The extractive value in alcohol was found to be 12.23±1.7, 14.8 ±1.4 and 15.2 ±0.11 %w/w respectively. The extractive value in water was found to be 30±2.4, 36±1.2 and 22.8 ±0.41%w/w respectively by soxhlet method.

5. Conclusion

In conclusion, the present study has shown that the investigation of polyherbal formulation of *aqueous* extracts of *Azadirachta indica, Camellia sinensis and ethanol extract Asparagus racemosus (N:G:S=2:2:1) 200 mg/kg2:2:1 named F1, (N:G:S=2:1:2) 200 mg/kg2:1:2 named F2, (N:G:S=2:1:1) 200 mg/kg2:1:1 named F3. was* carried out to certain findings about the pharmacognostical physiochemical and phytochemical features which no doubt can be proved beneficial and serve as scientific background for further isolationary steps to obtain the lead.

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