Physico-chemical and Phyto-chemical Standardization of 'Kanghi booti' (*Abutilon indicum* Linn.)

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Abstract

butilon indicum Linn. (Family: Malvaceae), commonly known as 'Kanghi booti' is a perennial herb, distributed throughout tropical India and Siri Lanka. The herb is used widely in many ailments such as fever, gonorrhoea, meningitis, chronic cough, diarrhoea and diseases of thorax. It is also used for eye wash. As per ethno-medical literatures the drug is reported as antiflatulant, antihelminthic, anti-inflammatory, demulcent and diuretic.

It is interesting to note that being an important plant, it is lesser known and except the positive antimicrobial activity on gram positive and gram negative bacteria, no works are reported in the literature. Therefore, an effort has been made to carry out the physicochemical and phytochemical studies of plant to find out the possible active constituent. The plant was collected directly from premises of Aligarh Muslim University Aligarh, in the month of December 2008. The successive extraction, soluble matter in aqueous and alcohol, ash values, loss on drying, moisture content, pH values, qualitative and quantitative analysis of organic constituents were estimated. The percentage of alkaloid (1.54%), nitrogen (0.02%), fatty matter (0.70%), phenols (0.27%), flavonoid (5.60%), sterols/terpenes (0.17%), proteins (1.02%), carbohydrate (1.04%) and crude fibre (2.75%) are reported. With the help of descending paper chromatography, amino acids and sugars were also identified and quantified.

Key words: Kanghi booti, Physicochemical and Phytochemical standardization, *Abutilon indicum* Linn.

Introduction

The drug consists of whole plant of *Abutilon indicum* Linn. (Family: Malvaceae), is reported in Unani System of Medicine to cure many ailments. As mentioned by Dymock *et al* (1890) another species of *Abutilon* is reported by Ibn-e-Sina (Avicena), grows at hilly areas, its fruits resemble to 'pumpkin' and the leaves are similar to the leaves of coriander. This species was confirmed as *Abutilon avicenna* Gartn. by Dymock *et al.* (1890). Ibn-e-Sina described it as wound healer. 'Kanghi booti' is written in various Arabic and Persian works under the name of "Masht-el-ghoul" and "Dieshar" respectively and mentioned that its bark is diuretic. Mostly the leaves of the plant are used for medicinal purposes but to get more beneficial effects the seeds and roots of the herb can also be used along with leaves and stem (Ghani, 1921).

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Abutilon is a large genus with record of 120 species (Anonymous, 1948; Kirtikar & Basu, 1995). It is distributed throughout tropical India and Sri Lanka. Leaves are cordate nearly entire or irregularly toothed covered on both surfaces with closely-felted white down with few or no hairs intermingled; sepals ovate; carpals 15-20 longer them the calyx, glabrescent, truncate or shortly awns, spreading; stipules deflexed, peduncles longer than the petioles jointed near the top; flowers yellow, 2.5 cm diameter, opening in the evening. (Hooker, 1875)

The drug is reported by various scholars as Antiflatulant, antihelminthic, antiinflammatory, antipyretic, aphrodisiac, astringent, analgesic, demulcent, styptic, diuretic, laxative and resolvent. It is useful in asthma, burning micturition, meningitis, chronic cough, diarrhea, diseases of thorax and also used for washing eye. It is used in gonorrhoea, haematuria, haemoptysis, inflamed gums, jaundice, leprosy, menorrhagia, bleeding pile and diseases of bile. The juice of leaves is beneficial in rabies, syphilis, stone in bladder and toothache. The fumigation of its seed is useful in ano rectal fistula and scariasis and ulcer (Khan, 1859; Khan, 1896; Ghani, 1921; Ghulam, 1923; Khan, 1937; Daljeet, 1974; Lubhaya, 1984; Anonymous, 2006; Munshi, 2007).

No work has been reported except the positive antimicrobial activity on gram positive and gram negative bacteria (Abdullah *et al*., 2010). Keeping in view the medicinal importance of this plant in Indian System of Medicine, a physico-chemical and phytochemical study of 'Kanghi booti' was carried out.

Materials and Method

The drug sample of "Kanghi" was collected from the premises of Aligarh Muslim University, Aligarh in the month of December 2008. With the help of available literatures 'Kanghi booti' was identified and confirmed as *Abutilon indicum* Linn. (Family: Malvaceae), the specimen deposited in the museum of the Department of Ilmul Advia (Voucher No.SC 0115/09). The whole plant of 'Kanghi booti' was air dried and ground to get coarse powder and then subjected to physicochemical and phytochemical studies. Ash values (total ash, acid insoluble ash and water soluble ash) and loss on drying were determined and estimated using the methods recommended in Indian Pharmacopoeia (Anonymous, 1996). The moisture content was determined by Toluene distillation method. The extractive values in petroleum ether (60-80^{o}c), diethyl ether, benzene, chloroform, ethyl alcohol and water were successively estimated using Soxhlet's Apparatus. The pH value of 1% and 10% aqueous solution was also measured. The water soluble and ethanol soluble matter were determined. The percentage of alkaloid, nitrogen, fatty matter, phenols, flavonoid,

sterols/terpenes, proteins, carbohydrate and crude fiber were determined quantitatively. The alkaloid fraction was separated (Paech and Tracey, *et al*, 1955) and the TLC of this fraction was made. The phenol, flavonoids and amino acid were separated using method of Paech and Tracey (1955), Sharma *et al*. (1991) and Tandon *et al*. (1970) respectively. The TLC of phenol and flavonoids fraction were made. The colour emitted by the powder, with various treatments under day light as well as Ultra Violet light (short and long wave length) was observed. Thin layer Chromatography (TLC) profile of the extracts in different solvents were determined using pre-coated silica gel (60 F254) aluminum plates (layer thickness 0.25mm), Descending paper chromatography for identification and quantification of Amino acids (Gowenlock, 1988) and sugars (Afaq *et al*., 1994) were also made.

Observation and Results

(a) Phytochemical Studies

The drug contains alkaloids, carbohydrates, flavonoids, glycoside, tannin, phenols, proteins, starch, saponin, sterols/terpenes, amino acids and resin.

The percentage of total alkaloids, flavonoids, phenols, nitrogen, fatty matter, sterols/terpenes, proteins and carbohydrates that were determined are depicted in table 1.

Table 1: Quantitative Estimation of chemical constituents

S.No.	Chemical constituent	Percentage (w/w)*1.54±0.05
1	Total Alkaloid	1.54 ± 0.05
2	Total Flavonoid	5.60 ± 0.05
3	Phenol	0.02 ± 0.05
4	Nitrogen	0.27 ± 0.05
5	Fatty matter	0.70 ± 0.05
6	Sterol/Terpenes	0.71 ± 0.05
7	Protein	1.02 ± 0.05
8	Carbohydrates	1.04 ± 0.05

*Note: Values are average of three experiments.

(b) Fluorescence Analysis of the Drug

The fluorescence analysis of the powder of *Abutilon indicum* Linn., was carried out after treatment with different reagents and chemicals the powder was observed under Day Light and U.V. Light and the colours emitted are recorded in table 2.

Table 2: Fluorescence Analysis of powdered drug of *'Kanghi booti'* chemical reagent

S. No.	Powdered drug	Day light	UV short	UV long
1	Powdered drug + Con. HNO_3	Orange	Green	Green
2	Powdered drug +Con. HCL	Green	Green	Black
3	Powdered drug + Con. H_2SO_4	Black	Black	Black
4	Powdered drug + 2% Iodine Sol.	Brown	Dark green	Black
5	Powdered drug + Acetic acid	Dark green	Green	Green
6	Powdered drug + 10% NaOH sol.	Green	Dark green	Black
7	Powdered drug + Acetic acid + H_2SO_4	Black	Black	Black
8	Powdered drug +10%NaHO + few drop of $CUSO_4$ sol.	Green	Dark green	Black
	Powdered drug +10%NaHO +Few drop of Lead acetate	Green	Green	Black
10	Powdered drug +Acetic acid+5%FeCl + H_2SO_4	Black	Dark green	Black
11	Powdered drug +5% FeCl	Green	Dark green	Black
12	Powdered drug +1NHCl	Straw water	Green	Black
13	Powdered drug +2NHCl	Light green	Green	Black
14	Powdered drug +1N H_2SO_4	Straw	Green	Black
15	Powdered drug +2N H_2SO_4	Light green	Green	Black

(c) Thin Layer Chromatographic

TLC profile of different extracts, extracted using soxhlet apparatus was studied and the Rf Values of spots visualized after treatment of various reagents were measured. The colours of the spots were also noted. The details are given in table 3 (Fig No.1-5).

(d) TLC of Alkaloid fraction (Fig. 6)

The alkaloid fraction was separated from chloroform extract and the TLC of that fraction was made using Pre-coated aluminum plates (silica gel (60 F 254), Thickness 0.25 mm).

Table 3: TLC evaluation of Successive extracts of '*Kanghi booti*'

Extract	Spraying reagent	Mobile phase	No. of spots	Rf Values & colour
1.Petroleum Ether	Day light Iodine Vapour Vaniline Sulphuric acid UV Long UV Short	Petroleum Ether: Di-ethyl Ether (1:1½)	11 09 08 05 07	0.17 (Y), 022 (Y), 0.26 (Lg), 0.3 (Lf), 0.34 (Lg), 0.37 (Dg), 0.46 (G), 0.47 (Y), 0.52 (Dg), 0.65 (Dg), 0.71 (Lg). 0.26 (Lg), 0.31 (Lg), 0.38 (Dg), 0.47 (Ly), 0.52 (Dg), 0.59 (Dy), 0.65 (Dg), 0.70 (Ly), 0.82(Dy). 0.05 (P), 0.10 (P), 0.38 (Lg), 0.55 (Dp), 0.62 (P), 0.68 (Dp), 0.82 (Lp), 0.97 (Dp). 0.22 (P), 0.32 (P), 0.38 (P), 0.53 (Dp), 0.67 (P). 0.22 (Y), 0.38 (Y), 0.47 (Ly), 0.52 (Y), 0.65 (Y), 0.71 (Ly), 0.77 (B).
2. Diethyl Ether extract	Day light Iodine Vapour Vaniline Sulphuric acid UV Long UV Short Day light Iodine Vapour UV Short	Petroleum Ether: Di-ethyl Ether (1½:1)	10 09 13 08 07 05 04 05	0.7 (G), 0.11 (Dg), 0.14 (Lp), 0.35 (Dy), 0.46 (Lg), 0.52 (Lg), 0.59 (Ly), 0.66 (Gy), 0.74 (G), 0.83 Dg). 0.05 (G), 0.11 (Dg), 0.42 (Dy), 0.52 (Yg), 0.59 (Yg), 0.59 (Yg), 0.66 (G), 0.74 (G), 0.83 (Dg), 0.91 (Y). 0.05 (G), 0.11 (G), 0.16 (P), 0.28 (Lg), 0.38 (Lg), 0.45 (Lg), 0.54 (Lp), 0.61 (G), 0.61), 0.67 (Dg), 0.73 (P), 0.77 (G), 0.84 (Dg), 0.94 (Gp). 0.07 (P), 0.12 (Dp), 036 (P), 0.54 (Dp), 0.60 (P), 0.66 (P), 0.74 (P), 0.84 (Dp). 0.07 (Y), 0.12 (Y), 0.39 (Y), 0.60 (Y), 0.66 (Dy), 0.74 (Lg), 0.84 (Dg). 0.08 (Y), 0.19 (G), 0.30 (Y), 0.39 (G), 0.52 (Dg). 0.19 (G), 0.30 (Y), 0.39 (G), 0.52 Dg). 0.08 (Y), 0.19 (Y), 0.39 (Y), 0.52 Dy).
3.Chloroform extract	Day light Iodine Vapour	Petroleum Ether: Di-ethyl Ether (1:1)	08 08	0.12 (G), 0.22 (G), 0.38 (Lg), 0.54 (G), 0.68 (Y), 0.72 (Y), 0.78 (G), 0.83 (Dg). 0.12 (G), 0.22 (G), 0.38 (Lg), 0.54(G), 0.68 (Y), 0.72 (Y), 0.78 (G), 0.83(Dg).
4.Benzene extract	Vanillin Sulphuric acid UV Short		07 08	0.06 (P), 0.12 (P), 0.22 (G), 0.65 (G), 0.69 (G), 0.74 (G), 0.77 (P). 0.06 (Y), 0.12 (Y), 0.22 (Y), 0.51 (Y), 0.66 (Y), 0.71 (Dy), 0.80 (Dy).
5.Alcoholic extract	Day light Iodine Vapour UV Long UV Short	Chloroform: Methnol (1:¼)	05 05 01 03	0.40 (G), 0.44 (Dg), 0.49 (Y), 0.58 (Lg), 0.60 (Dg). 0.40 (G), 0.44 (Dg), 0.49 (Y), 0.58 (Y), 0.60 (Dg). 0.61 (P). 0.44 (Y), 0.49 (Y), 0.60 (Dy).

Note: G = green, Dg = dark green, Y = yellow, Dy = dark yellow, Lg = light green, Gy = greenish yellow Gp=grayish purple,

P = purple, Lp = light purple, Dp = dark purple

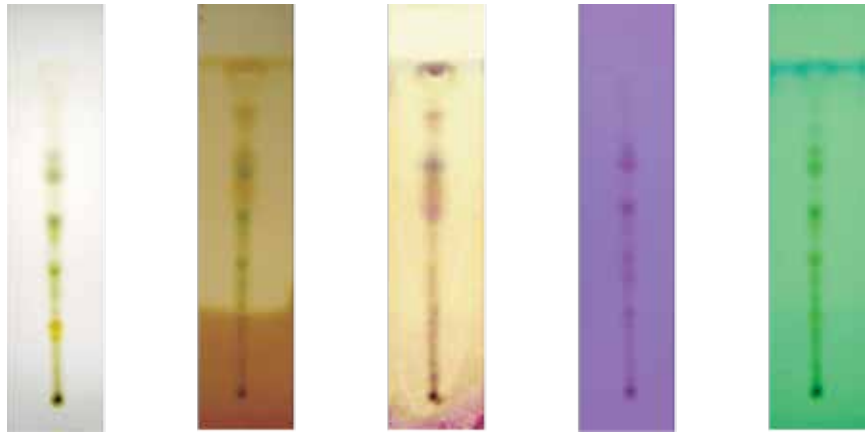

Fig. 1: TLC of Petroleum ether extract of *Abutilon indicum* Linn.

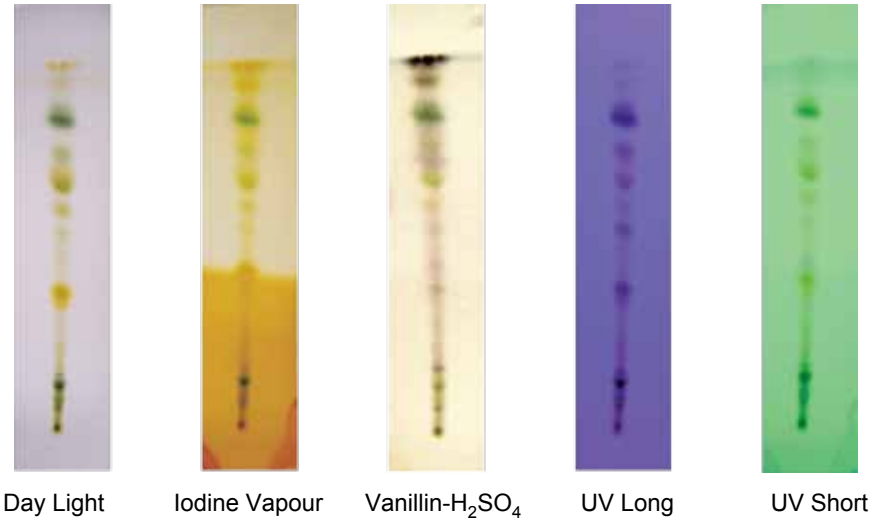

Fig. 2: TLC of Diethyl Ether extract of *Abutilon indicum* Linn.

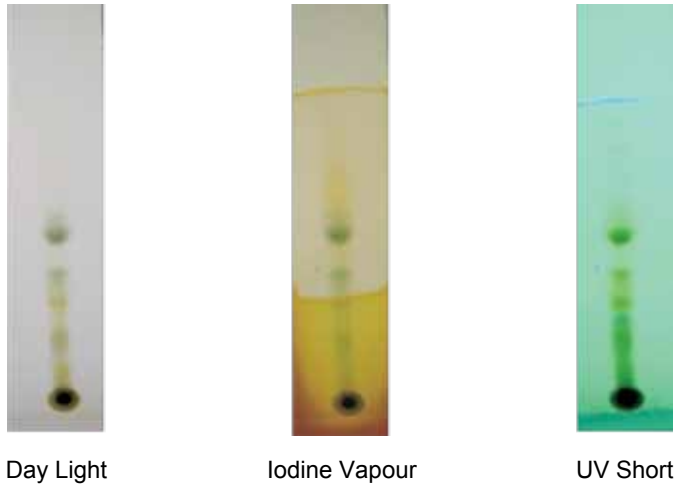

Fig. 3: TLC of Chloroform extract of *Abutilon indicum* Linn.

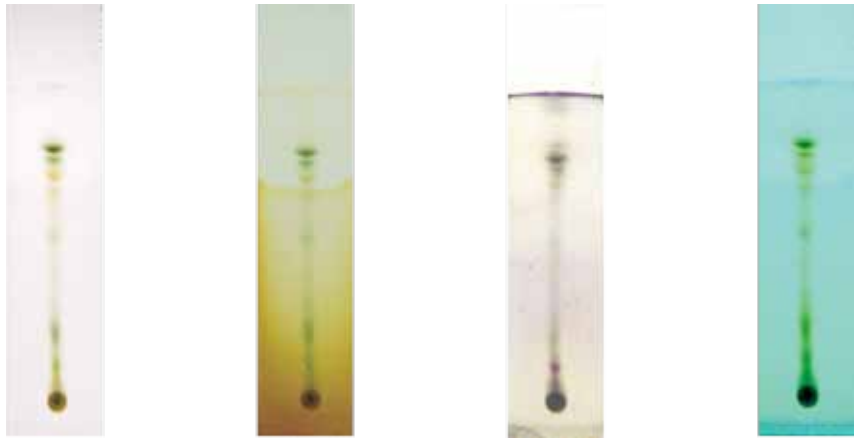

Fig. 4: TLC of Benzene extract of *Abutilon indicum* Linn.

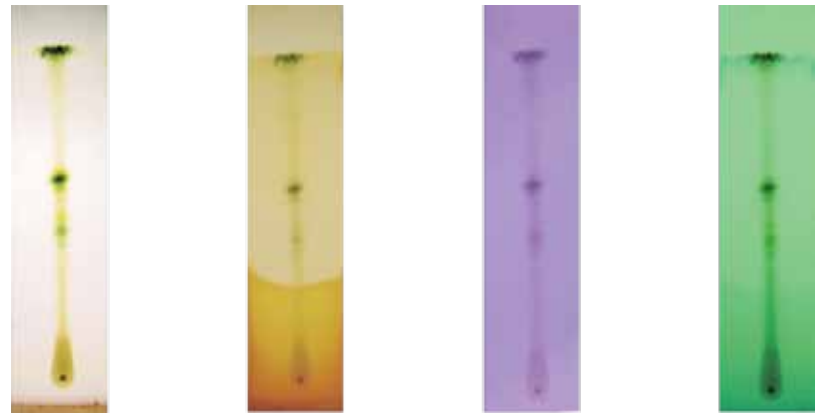

Fig. 5: TLC of Alcohol extract of *Abutilon indicum* Linn.

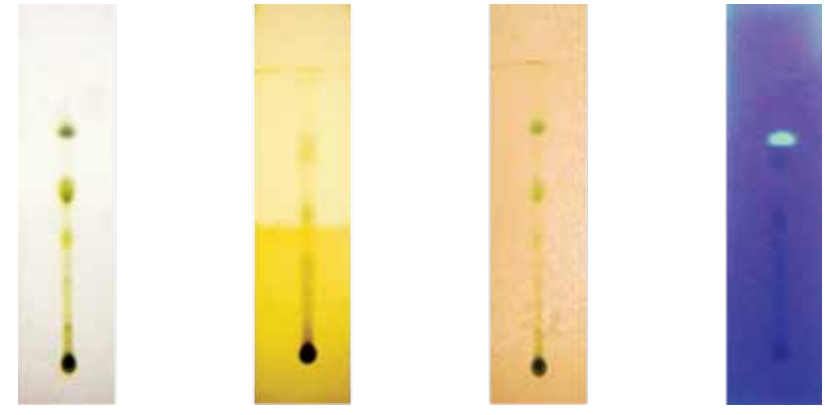

Fig. 6: TLC of Chloroform extract for alkaloid determination of *Abutilon indicum* Linn.

The Mobile Phase was Petroleum ether: Di-ethyl ether (1:1). In daylight 5 spots (Rf: 0.14, 0.30, 0.44, 0.62, 0.83) were clearly visible and in Iodine vapor 2 spots, (Rf: 0.48, 0.71) were visible. Using Vanillin Sulphuric acid as spraying reagent 5 spots (Rf: 0.14, 0.30, 0.44, 0.62, 0.83) was very clear, whereas under UV (long wavelength), 1 spot (Rf: 0.79) was visible.

(e) TLC of Flavonoides fraction (Fig. 7)

The TLC of flavonoid fraction on Pre-coated aluminum plates (silica gel (60 F 254), Thickness 0.25 mm) was made using Toluene, Ethyl acetate and Formic acid (50:40:10) as mobile phase. After spraying the plate with Polyethylene glycol, 3 spots (Rf: 0.45, 0.48, 0.76) appears, shows that three type of flavonoids are present in the plant.

5% Ferric chloride

Fig. 7: TLC of Ethanolic extract of *Abutilon indicum* Linn., for phenols determination

(f) TLC of Phenols fraction (Fig. 8)

The TLC of phenol fraction on Pre-coated aluminum plates, silica gel 60 F 254, (Thickness 0.25 mm) were made using Toluene and Ethyl acetate (8:2) as mobile phase. After spraying the plate with 5% Ferric chloride: 3 spots, (Rf: 0.26, 0.44, 0.49) appears indicate the presence of at least three phenolic compounds.

(g) Descending Paper chromatography for Amino acids:

Using Whatman filter paper (No.1). The amino acid fractions were subjected to decending paper chromatography. The mobile plate was the organic layer of n-Butanol, Acetic acid and Water, (6:2:2). The paper was sprayed with Ninhydrine solution (1% in acetone). Eight (8) amino acid were identified and

the percentage composition calculated using Torhniwal Densitometer and given in table no. 4.

Polytheleneglycol

Fig. 8: TLC of 85% and 50% methanol extract of *Abutilon indicum* Linn., for Flavonoid determination.

Table 4: Amino Acids and Sugars of 'Kanghi booti'.

Name	Percentage Composition
Amino Acids	
Cystine	4.50
Histidine	6.33
Serine	22.50
Alanine	10.60
Histidine hydrochloride	5.40
Tryptophan	6.40
Proline	8.60
Phenyl alanine	35.50
Sugars	
Glucose	50
Fructose	50

(h) Descending Paper chromatography for Sugars:

Using Whatman filter paper (No. 1). The alcoholic extract was subjected to descending paper chromatography. The n-Butanol, Acetic acid and Water

(4:1:5) organic layer, was selected as mobile phase. The paper was sprayed with Aniline phthalate solution. Two sugar spots identified. The percentage compositions are given in table no. 4.

Discussion

The present study is an attempt to ascertain the pharmacopoeial standards for the standardization of 'Kanghi booti'. The quality, identity, purity and strength of the powder has been undertaken as a tool to bring out several features like ash standards, solubility in alcohol and water, successive extractive values, and qualitative screening of physicochemicals, total alkaloids, total flavonoids, phenol, nitrogen, fatty matter, sterol/terpenes, protein, carbohydrates, amino acids and sugars. All these parameters could be incorporated as standards in Unani Pharmacopoeia. Characterization of an herbal drug is essential for the quality control to check the presence of adulterants as a single drug remedy or its polyherbal Unani formulation.

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