Quality Control Standardization and *In-Vitro* Antioxidant Activity of *Aganosma dichotoma* K. Schum Root

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ABSTRACT

Introduction: *Aganosma dichotoma* K. Schum (AD) is a large climber with very stout stem belonging to the family Apocynaceae. The Plant has significant medicinal value as described in traditional system of medicine. The objective of the present study is to scientifically develop a standard monograph for AD on the basis of its pharmacognostical and phytochemical aspects. **Methods:** The study includes quality control standardization as per the standard methods provided in World Health Organization for standardization of medicinal plants. Fluorescence drug analysis, preliminary phytochemical screening of different fractions, quantification of some phytoconstituents and in-vitro antioxidant activity were also carried out. Quantification of Quercetin in the ethanolic extract of *A. dichotoma* was determined by HPTLC analysis. The ethanolic extract of root of *A. dichotoma* was subjected to in-vitro antioxidant activity. **Results:** The diagnostic characters of *A.dichotoma* root were evaluated on the basis of macroscopical and microscopical characters. Physicochemical parameters were evaluated such as 6.7% w/w loss on drying with; ash values (in % w/w): 13.75 total ash, 5.75 acid-insoluble ash, 3.6 water-soluble ash; Extractive values (% w/w): 12.75 water, 11.82 ethanol, 2.26 ethyl acetate, 3.13 chloroform, and 3.16 pet ether; foaming index 181.81; swelling index 3.2 ml/g; hemolytic activity 227.89 unit/gm of powder drug and crude fiber content was 19.4%. Total numbers of starch grain in 1 mg of root powder were 2,49,981. Quantification of quercetin in the ethanolic extract was assessed by HPTLC analysis and was found to contain 2.40%, w/w. **Conclusion:** The parameters determined in the present study may provide necessary information for identification and authentication of plant material.

Key words: *Aganosma dichotoma*, HPTLC, *in-vitro* antioxidant activity, Pharmacognosy.

INTRODUCTION

From time immemorial plants, parts of plant and isolated phytochemicals have been used in prevention and treatment of various health ailments as different formulations especially in traditional system of medicine like Ayurveda, Siddha and Unani.[1] According to the World Health Organization (WHO), almost 80% of the world's population depends on traditional medicine as their primary healthcare needs.[2] The active researches in the field of herbal traditional medicines have gained considerable momentum worldwide during the past decade. A part from incredible therapeutic potentials, herbal drugs formulations are prone to contamination, deterioration and variation in composition of constituents, which gives rise to little or no therapeutic efficacy.[3] In order to obtain good quality herbal formulations, it becomes extremely important to make an effort towards standardization of the plant material (used as traditional medicine) for proper marketing authorization and approval.

A. dichotoma is commonly known as Malati in Hindi and in Sanskrit it is known as Jati. Eight species of *A. dichotoma* are widely distributed from India to China Philippines and Indonesia. In India, it is mainly distributed across Assam, Bihar, West Bengal, Orissa, Andhra Pradesh and Tamil Nadu.[4] The plant is a large climber with very stout stem and belongs to family Apocynaceae. This plant produces milky latex which is a characteristic of Apocynaceae family. *Aganosma* was first described by Blume (1826) as a section of Echites and raised to generic status by G. Don (1837) with a number of species based on specimens in Wallich's herbarium.[5] Traditionally, *A. dichotoma* is used as emetic, anthelmintic, in bronchitis, leprosy, skin diseases, ulcer,

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inflammations, arthritis, purulent discharges from the ear and diseases of the mouth. Flowers are good for eye diseases and leaves cure biliousness.[4] Some reported phytoconstituents in flowers includes β-sitosterol, ursolic, vanillic and ferulic acids, quercetin and its glycosides, rutin, hyperin, isoquercetin and quercetin-3-arabinosides.[6] Leaves contain quercetin, kaempferol, glycoflavones, leucoanthocyanins and vanillic, syringic, protocatechuic, ferulic and sinapic acid.[7] Even though the plant is used traditionally for ailment of various diseases, however, there is no data available on its quality control profile.

The basis of nomenclature system used in ancient time attributing different names to the same plant and same name for different plants together with the time, have caused considerable confusion in selection of medicinal plants in various parts of the country. One of the examples is **Parpat** which is important drug of the Indian system of medicine. Nine different plants i.e., *Fumaria indica* (Fumariaceae), *Polycarpaea corymbosa* (Caryophyllaceae), *Justicia procumbens* (Acanthaceae), *Rungia repens* (Acanthaceae), *Rungia parviflora* (Acanthaceae), *Peristrophe bicalyculata* (Acanthaceae), *Glossocardia linearifolia* (Compositae), *Mollugo stricta* (Ficoidaceae) and *Oldenlandia corymbosa* (Rubiaceae) are known as parpat but all of them are totally different from each other and having different medicinal uses.[8-10] Vernacular name of *A. dichotoma* is Malati in Hindi and there are some other plants which are also known as Malati in different languages, for example *Combretum indicum* (Combretaceae) known as malati in Hindi[11] and *Jasminum grandiflorum* (Oleaceae) (Malati in Telugu).[12] Due to same common name of the medicinal plants there are chances of misidentification resulting in ambiguity. Hence standardization will provide guideline for proper identification. Keeping this in view was performed pharmacognostical standardization of *A. dichotoma* root.

MATERIALS AND METHODS

Plant Material

A. dichotoma roots were collected from Tumbura Kona Kshetram at Seshachalam hills and Tirumela hills, Chittor District, Andhra Pradesh, South India in the month of April. It was authenticated by Dr. K. Madhava Chetty, Taxonomist, S. V. University, Tirupati. A voucher specimen (COG/AD/17) has been retained in Department of Pharmaceutics, Indian Institute of Technology-BHU, Varanasi, India for further reference.

Macroscopic and Microscopic Evaluation

The dried roots were studied for their macroscopic character such as color, odour, taste, size, shape and texture. The roots were cleaned and fixed in formalin, acetic acid and 70% ethanol mixture for 24 hr prior to experimentation. The specimens were dehydrated with graded series of tertiary butyl alcohol.[13] Infiltration of specimen was carried out by gradual addition of paraffin wax until the solution attained super saturation. Then the specimens were cast into paraffin blocks and sectioned with the help of rotary microtome (at 10 μm to 12 μm thickness) followed by dewaxing.[14] Finally the sections were stained with toludine blue, and phloroglucinol & HCl solution (1:1). Sections were photographed with a Nikon trinocular microscopic unit, Model E-200, Japan. For powder microscopy, fine powder of root was cleared with chloral hydrate and stained with phloroglucinol and conc. HCl solution (1:1) and mounted with glycerin.

Determination of Physicochemical Parameter

From dried root the presence of any foreign matter was resoluted manually by using magnifying lens. Some more physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, loss on drying, extractive value in different solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water), foaming index, swelling index and hemolytic activity of *A. dichotoma* were determined. The crude fiber content was determined by boiling the plant material with 10% nitric acid and treating it with 2.5% NaOH solution.[15] Total number of starch grains in *A. dichotoma* root was estimated by using the lycopodium spore method.[16] Fluorescence analysis is an important qualitative diagnostic tool for the presence of chromophore in crude powdered drug. Fluorescence powder drug analysis was performed under daylight, short UV, and long UV.[17]

Preliminary Phytochemical Screening

The coarsely powdered root of *A. dichotoma* was extracted by hot soxhlation using 95% ethanol (3L v/v) as solvent for extraction till the powdered drug was exhausted. The resulting extract was filtered and concentrated under reduced pressure to obtain the crude ethanolic extract of *A.dichotoma* (EAD). After the extraction process, successive fractionation was done by suspending the ethanolic extract (EAD) in aqueous media then partitioning with solvents of varying polarity such as Petroleum ether, chloroform

and ethyl acetate in order of their ascending polarity. Further, the extract (EAD) and its successive fractions were subjected to preliminary phytochemical tests to check the presence of various phytochemical classes.[18]

Quantitative Estimation of Phytoconstituents

Total Phenolic content

Total phenolic content present in the ethanolic extract of root of *A. dichotoma* was estimated by using Folin ciocalteau reagent method.[19] In a test tube aliquots of varying concentration of the extract were taken and volume was made up to 1 ml with distilled water. 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Then the tubes were vortexed, placed in the dark for 40 min and the absorbance was recorded at 725 nm. The amount of total phenolics was calculated as tannic acid equivalents from the calibration curve.

Total Tannin content

Tannins are separated from rest of the mixture by adsorption on insoluble matrix polyvinylpolypyrrolidone (PVPP), and the total tannin content was determined by Folin–Ciocalteu procedure (mentioned as above). Insoluble, cross-linked PVPP (100 mg) was taken in a test tubes and 1.0 ml distilled water and 1 ml tannin containing extract was added. Tubes were maintained at 4 °C for 15 min, then subsequently vortexed and centrifuged for 10 min and the supernatant was collected. Aliquots of supernatant (0.2 ml) were transferred into test tubes, and then non-absorbed phenolics were determined. Finally observed values were subtracted from total polyphenol contents, and the total tannin content is expressed as; mg Tannic acid/100 g dry plant material. All measurements were done in triplicate.[20]

Total Flavonoid and Flavonol content

In the ethanolic root extract total flavonoid content was estimated by using aluminum chloride, standard compound Rutin was used as reference. The method for the estimation of flavonoids content is based on the formation of a flavonoid-aluminium complex (λmax 415 nm). In a test tube 1 ml of alcoholic plant extract, 1 ml of aluminium chloride and 3 ml of sodium acetate were mixed. After 2.5 hours, absorbance was measured at 415 nm.[21] The total flavonoid and total flavanol content were estimated by formula;

$$X = (A.m_o) / (A_o.m)$$

Here

X: the flavonoid content, mg/gm plant extract in root extract

A: the absorption of plant extract solution

A_o: the absorption of standard solution

m: the weight of plant extract (mg)

m_o: the weight of Rutin in solution (mg)

Total Alkaloid content

Total alkaloid content was estimated by gravimetric method.[22] 5 gm powdered drug was extracted repeatedly with 0.1N H_2SO_4 in an ultrasonic bath (3x50 ml). Then the solution was filtered and washed the acidic solution with chloroform in four successive quantities of 25 ml. The chloroform washing was rejected and acid solution was basified with dilute ammonia solution and then extracted with diethyl ether (20 ml x 5). Diethyl ether extract was washed with 5 ml distilled water, and then residue was dried to constant weight at 105^{0}C.

Total Saponins content

Total saponin content was estimated using Diosgenin as reference compound. The plant material was initially centrifuged with aqueous methanol and subsequent treatment with anisaldehyde-ethyl acetate reagent and H_2SO_4 and finally its absorbance is measured at 430 nm.[23]

Pesticide Residue

For estimation of pesticides like (aldrin, HCH, DTH, dieldrin, and malthion *etc.*) in crude drugs, Florisil R (grade 60/100) column chromatographic technique was used. Acceptable daily intake of aldrin and dieldrin is not more than 0.0001mg/kg body weight. As per the WHO guidelines for determination of pesticide residues, HCH and DTH should be no more than 0.005 mg/kg body weight.[15]

WHO and FAO (Food and Agricultural Organization) set the maximum permissible limits of pesticides frequently present in the herbs. Most of these pesticides are mixed with the herbs during the time of cultivation. Usually pesticides like DDT, BHC, toxaphene, aldrin cause various serious side-effects in human beings. The pesticide content

of the powdered root of *A. dichotoma* was determined by treating with acetonitrile: water (65:35) mixture followed by blending and filtration. To the filtrate (250 ml), 100 ml light petroleum, 10 ml sodium chloride (40%) and 600 ml water were added with constant shaking for 35 to 45 sec. The solvent layer was separated and washed twice with 100 ml portions of water to which 15 g of anhydrous sodium sulfate was added with vigorous shaking. The extract was separated, and its volume was reduced to 5 ml to 10 ml, this extract is then passed through column (packed with Florisil R grade 60/100 PR, activated at 650^{0}C). Three elutes were obtained after running the column with three ratios of ether: light petroleum mixture containing 6%, 15%, and 50% of ether, respectively. Elutes were evaporated to dryness, transferred to a sample holder, and burned in a combustion flask flushed with oxygen containing the solvent (water for chloride and H_2SO_4 in case of phosphate pesticides). For determining the chloride pesticides, 15 ml of this solution was mixed with 1 ml of ferric ammonium sulfate (0.25 mol/l) and 3 ml of mercuric thiocyanate and allowed to stand for 10 min after swirling and absorbance was measured at 460 nm. Phosphate pesticides were determined after mixing 7 ml solution to 2.2 ml sulfuric acid (300 g/l), 0.4ml ammonium molybdate (40g/l), and 0.4 ml aminonaphtholsulfonic acid followed by swirling, heating at 100^{0}C for 12 min, and absorbance was measured at 820 nm.[15]

Quantification of Quercetin in A. dichotoma by HPTLC

Ethanolic Extract of root of *A. dichotoma* (EAD) was standardized with quercetin using high performance thin layer chromatography (HPTLC). A stock solution of EAD (10 mg/mL), and quercetin (0.2 mg/mL) was prepared in methanol. The mobile phase for developing the chromatogram consisted of chloroform, methanol and formic acid mixture in the ratio 75:15:10 (v/v/v). The study was carried out using Camag- HPTLC instrumentation (Camag, Mutten, Switzerland) equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer and WINCATS 4 software for data interpretation. The R_f values was recorded and the developed plate was screened and photo-documented at ultra violet range with wavelength (λmax) of 254 nm.

In-vitro Antioxidant Study

In-vitro antioxidant activity of extract was evaluated due to presence of phenolic and flavonoid compounds in extract. The ethanolic extract of root of *A. dichotoma* was then subjected to *in-vitro* antioxidant activity by using; DPPH method, total antioxidant capacity, scavenging of hydroxyl radical by deoxyribose method, scavenging of nitric oxide radical, assay of reducing power and scavenging of hydrogen peroxide. DPPH assay method is based on the reduction of ethanolic solution of colored free radical DPPH by free radical scavenger. Absorbance was measured at 517 nm and ascorbic acid was used as standard.[24] Total antioxidant capacity of ethanolic extract of plant was evaluated by using phosphomolybdenum. This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo(V) complex at acidic Ph.[25] The hydroxyl radical scavenging activity of plant extract was evaluated by deoxyribose method using butylated hydroxyl anisole (BHA) as a standard and absorbance was measured at 532 nm.[26]

In nitric oxide scavenging assay; sodium nitroprusside in aqueous solution at physiological pH spontaneously generated nitric oxide, which interacted with oxygen to produce nitrite ions, that can be estimated by using modified Griess Ilosvay reaction and the absorbance of chromophore formed was measured at 540 nm.[27] Potassium ferricynide method was used for the assay of reducing power using ascorbic acid as standard and absorbance was measured at 700 nm. H_2O_2 scavenging activity of plant extract was evaluated, and absorbance was recorded at 230 nm and percent inhibition calculated.[28]

RESULTS

Macroscopic and Microscopic evaluation

Dried roots are cylindrical with slightly tapered in shape,

Figure 1: *Aganosma dichotoma* G.DON root

6-10 cm long and 0.5-1.5 cm in width. Outer surface of root is dark reddish brown, much shriveled and wrinkled longitudinally while internally root is buff to light yellow in color. Outer layer is easily exfoliated, separating from the wood in large, papery flakes or strips (Figure 1). It has no perceptible odor and taste.

Transverse section of *A. dichotoma* root shows exfoliating wavy cork, consisting of 3-4 layered brick shaped cork cells filled with tannins (Phellem). Next to phellem, phelloderm is present, made up of 8-10 layered parenchymatous wide cells. Single layered pericyclic sclerenchymatous cells are situated in between the phellem and phelloderm. Periderm is followed by secondary cortex which is made up of 10-12 layered thin walled parenchymatous cells. A number of parenchymatous cells of secondary cortex contain starch grains, which are simple, round to oval in shape but the hilum and striation are indistinct. Solitary calcium oxalate crystals are also found in cortex region. Anomalous structures are reported, formation of xylem and phloem is irregular, which shows furrowed xylem and phloem is situated in furrows. The secondary xylem consists of narrow vessels and vasicentric tracheids, both having simple pits. The medullary rays become more deep and monoseriate in the section. Additional arcs of phloem more deeply seated in the pith known as intraxylary phloem which is a main characteristic of *Aganosma* (*Echites*) genus. Pith is frequently containing sclerosed elements (Figure 2).

The macerated powdered characteristics of *A. dichotoma* roots were expressed in terms of µm (minimum-mean-maximum; length x width) and showed the presence of a large number of fibres having slender shape and tapering ends with septa (131.4–158.45-183.55 x 3.6-5.4-7.2 µm), tracheids are pitted thickening with tapering ends, measuring (102.90-126.35-149.80 x 4.7-6.5-8.3 µm), xylem vessels of varying size and shape measuring (58.23-72.79-97.85 x 17.90-24.67-28.90 µm) and have pitted thickenings. Group of cork cells and lignified sclerenchymatous cells were appeared in the powder microscopy. Parenchymatous cells were also visible which are round, oval and elongate in structure having size varying between (24.62-31.81-67.42 x 15.90-24.63-37.67). Solitary calcium oxalate crystals with varying size and shape (25.48-35.67-36.18) were also present (Figure 3).

Physicochemical Parameters

The plant material had 0.837% w/w foreign matter and showed 6.7% w/w loss on drying with; ash values (in % w/w): 13.75 total ash, 5.75 acid-insoluble ash, and 3.6 water-soluble ash; Extractive values (% w/w): 12.75 water, 11.82 ethanol, 2.26 ethyl acetate, 3.13 chloroform, and 3.16 pet ether; foaming index 181.81; swelling index 3.2 ml/g; hemolytic activity 227.89 unit/gm of powder drug and crude fiber content was 19.4%. Total numbers of starch grain in 1 mg of root powder were 2,49,981. Fluorescence powder drug analysis of root of *A. dichotoma* varied with

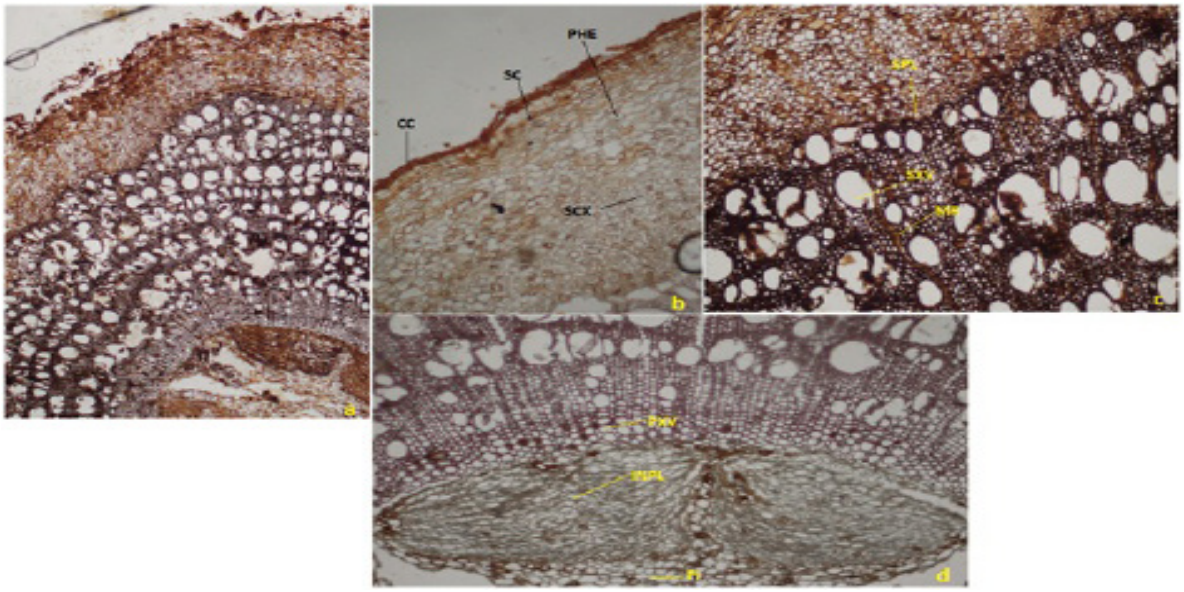

Figure 2: Histological study of A.dichotoma root. [a]: Transverse section of root [b]: T.S showing Periderm Region [c]: T.S. showing Secondary Phloem and Secondary Xylem [d]: T.S. showing intraxylary Phloem and Pith region [CC –cork cell layer, SC- Sclerenchymatous cell layer, PHE- Phelloderm, SCX- Secondary Cortex, SPL- Secondary Phloem, MR- Medullary Rays, SXV- Secondary Xylem Vessel, PXV- Primary Xylem Vessel, INPL- Intraxylary Phloem, PI- Pith

TABLE 1: Fluorescence Analysis of A. dichotoma Root Powder Drug

Test	Day light	Short UV	LongUV
Powder + 1 N NaOH in methanol	Peach Puff	Light green	Honey dew
Powder + 1 N NaOH in water	Dark golden rod	Green yellow	Lime green
Powder + 1 N HCL in methanol	Saddle Brown	Yellow green	Daark Salmon
Powder + 1 N HCL in water	Rosy Brown	Dark sea green	No Fluorescence
Powder + 1 N HNO3 in methanol	Wheat color	Lawn green	Light green
Powder + 1N HNO3 in water	Tan	Light green	No fluorescence
Powder + 5% iodine	Maroon	Dark red	No fluorescence
Powder + 5% FeCl3	Sienna	Dark green	No fluorescence
Powder + 50% KOH	Khaki	Yellow green	Lime
Powder + 25% ammonia	Corn silk	Green yellow	Olive drab
Powder + picric acid saturated	Gold	Green yellow	No fluorescence
Powder + acetic acid	Antique white	Dark sea green	Dark olive green

TABLE 2: Preliminary Phytochemical Screening of Ethanolic Extract of A. dichotoma and its Successive Fractions:

Phytoconstituents	EAD	PEF	CF	EAF	AF
Flavanoids	+	-	-	-	-
Phenolics & Tannins	+	-	-	-	+
Steroids	+	+	+	+	-
Coumarins	-	+	+	+	-
Cardiac Glycosides	+	-	-	-	+
Anthraquinone Glycosides	+	-	-	-	-
Alkaloids	-	-	+	-	+
Saponin	-	+	-	-	+
Carbohydrate	+	-	+	+	+
Reducing Sugar	+	-	+	+	-

(EAD- Ethanolic extract of A. dichotoma root powder, PEF-Petroleum ether fraction, CF-Chloroform fraction, EAF-Ethyl acetate fraction, AF-Aqueous fraction)

TABLE 3: Quantitative Estimation of Phytoconstituents of Ethanolic Extract of Aganosma Root

Phytoconstituent Class	Total Content in mg/gm of Plant Extract
Total Phenolic Content	125.65 ± 2.58 (Equivalent to Tannic Acid)
Total Tannin Content	104.96 ± 1.35 (Equivalent to Tannic Acid)
Total Flavanoid Content	62.20 ± 2.01 (Equivalent to Rutin)
Total Flavanol Content	1.97 ± 0.06 (Equivalent to Rutin)
Total Alkaloid Content	0.2% w/w
Total Saponin Content	49.2 ± 1.92 (Equivalent to Rutin)

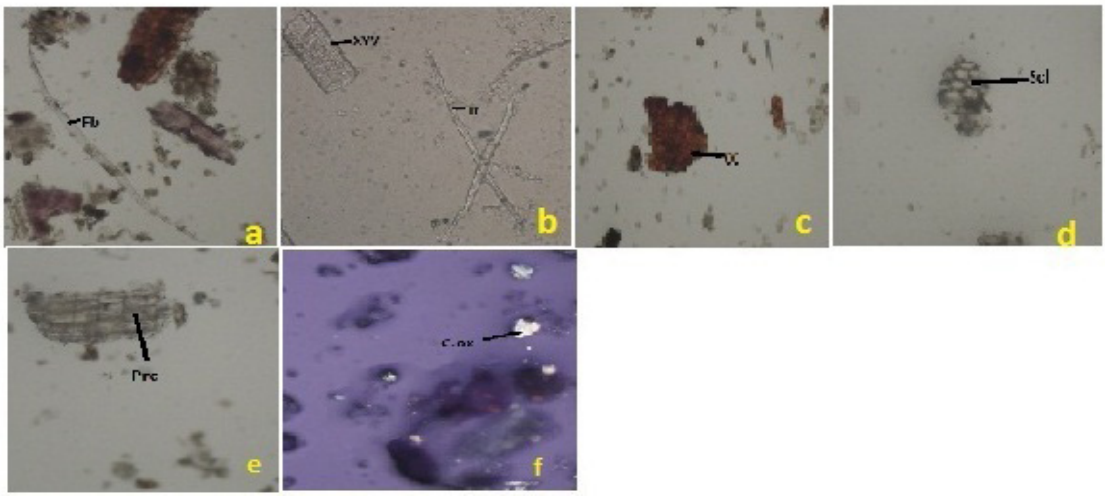

Figure 3: Powder characteristics of Aganosma root powder, [a]: Fiber (Fb), [b]: Pitted Xylem Vessel (XYV) and Tracheids (Tr), [c]: Cork Cells (CC), [d]: Sclerenchymatous Cells (Scl), [e]: Parenchymatous Cells (Prc), [f]: Solitary Calcium Oxalate Crystals (C.ox)

the extract (Table 1).

Preliminary Phytochemical Screening

The extracts of root of *A. dichotoma* varied in the phytochemicals profile (Table 2).

Quantification of Phytoconstituents

Quantification of different phytoconstituents (Total phenolic, Total tannin, Total flavanoid, Total flavanol, Total alkaloid and Total saponin) were reported in (Table 3).

Determination of Pesticide Residue

Chlorinated pesticide obtained in first and second elute from column were 0.1426 and 0.2794 mg/kg of the crude drug respectively. Phosphated pesticide obtained in first, second and third elute from column were 0.0032, 0.0215 and 0.0107 mg/kg of the crude drug respectively.

Quantification of Quercetin in A. dichotoma by HPTLC

HPTLC study for the quantification of quercetin in EAD was analyzed in Fig 4 for the first time by scanning at wavelength (λmax 254 nm). Quantity of quercetin present in EAD was found to be 2.40% (w/w).

Determination of In-vitro Antioxidant Study

The total antioxidant capacity of ethanolic extract of root of *A.dichotoma* showed potential antioxidant activity compared with the Ascorbic acid used as a standard. The absorbance of ethanolic extract of plant (100 µg/ml) was 0.356 at 695 λ_{max}. Thus the result of Total antioxidant capacity was found to be 57.75 µg/ml of ethanolic extract of *A.dichotoma* which was equivalent to 100 µg/ml of standard. Ascorbic acid. The result of DPPH scavenging activity in this study indicates that the plant was potentially active. Ethanolic extract showed IC_{50} value (104.41 ± 0.2) while standard. Ascorbic acid showed IC_{50} value (98.20 ± 4.11). Nitric oxide scavenging activity was estimated by using Griess reagent, which showed a very moderate scavenging activity of plant extract (IC_{50} 137.07 ± 0.72) in comparison to rutin (IC_{50} 71.32 ± 1.91). The scavenging potential of hydrogen peroxide by ethanolic extract was also found to be considerably moderate with IC_{50} value of 143.80 ± 0.81 compared to rutin (IC_{50} 99.92 ± 1.18). Hydroxyl radical production was assessed by the iron (II)–dependent deoxyribose damage assay following the Fenton reaction. The result demonstrated a very good scavenging activity with an IC_{50} value of 127.63 ± 0.56 compared to positive control BHA (IC_{50} 103.14 ± 2.53). Assay of reducing power is a concentration-dependent reaction. The assay of reducing power depicted a very moderate reducing capacity of the extract (0.198 ±0.01 µg/mL) as compared to standard ascorbic acid (0.419 ± 0.006 µg/mL).

DISCUSSION

Currently, there is a great demand on the quality control profile and standardization of medicinal plant drug/plant

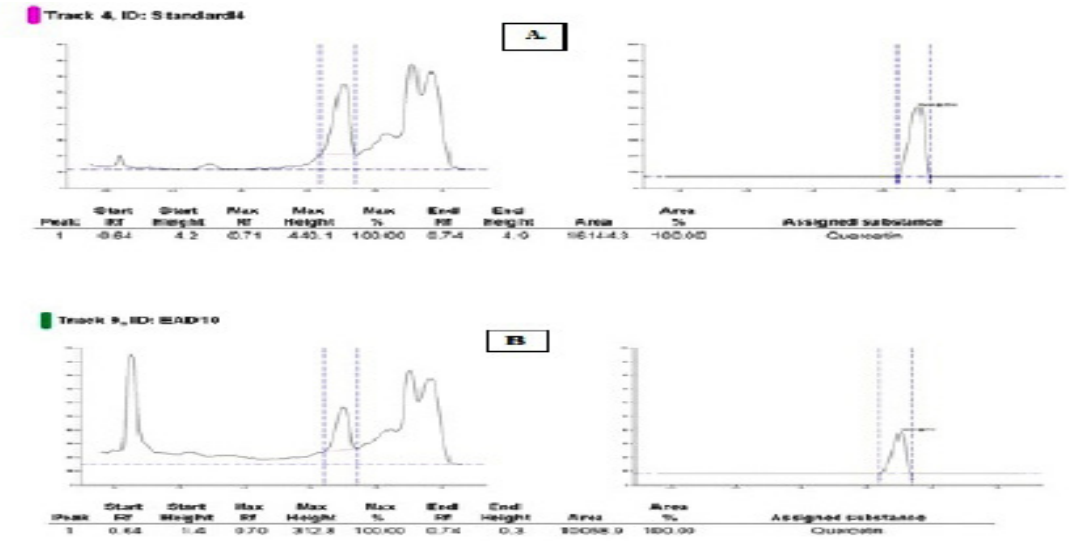

Figure 4: HPTLC densitogram of quercetin in ethanol extract of A.dichotoma (EAD). In figure A: Standard peak of quercetin B: Peak of quercetin present in EAD

parts for their therapeutic potentials. According to the World Health Organization, modern pharmacognostic techniques are available for the identification and evaluation of crude drugs which are more reliable, accurate and inexpensive. Pharmacognostical evaluation of a particular drug of a plant/plant parts provides valuable information in terms of its morphological, microscopical, and physical characteristics and is therefore, preferred as a primary step in standardization of a plant. Therefore some diagnostic features have been evolved to identify and to differentiate *A. dichotoma* root from the other crude drugs and its adulterants. From the microscopical studies, it was observed that the T.S. of root showed the presence of various unique indicative characters which are the intraxylary phloem, solitary calcium oxalate crystals *etc.*

The physicochemical evaluation is an imperative parameter which is useful in detection of adulteration or improper handling of the drug. The significance of ash values are quantitative standards that correspond to the presence of various impurities like carbonate, oxalate and silicate which may be naturally occurring or intentionally added to crude drug as a form of adulterant. Total ash includes both physiological as well as non–physiological ash, while acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs.[29] Extractive values are valuable to evaluate the amount of active chemical constituents present in the plant/plant parts using different solvents. Loss on drying indicates that the drug is safe regarding any growth of bacteria, fungi and yeast.[30] The foaming index parameter is the ability of plant material and their extracts to form importunate foam. The swelling index is carried out due to therapeutic or pharmaceutical value of many medicinal plants may be attributed to its swelling property, which is due to the presence of gums, mucilage, pectin, and hemicelluloses. Hemolytic activity is seen in plants containing mainly saponins that diffuse hemoglobin into the surrounding medium[15]. Preliminary phytochemical screening revealed the presence of anthraquinone and cardiac glycosides, alkaloids, flavonoids, tannins, steroids, saponin and coumarins in different fractions of *A. dichotoma*. Such phytochemical screening is helpful in the prediction of natural phytoconstituents present in the tested drugs, which further leads to the isolation of compounds since phytochemicals are proven to be responsible for the activity of the drugs. Quantitative estimation of phytoconstituents is efficient parameters to set up standards for crude drugs. Moreover, the chemical standardization of EAD was also ascertained with the help of HPTLC and the amount of quercetin was quantified as a chemical marker.

The results obtained from the *in vitro* antioxidant studies showed that ethanolic extract of *A. dichotoma*, possess potent antioxidant activity, which may be attributed to the good availability of phenols, tannins, and flavonoids. The total antioxidant capacity and reducing power of a plant play a dominant role in depicting its antioxidant activity. All these plant based constituents should be well standardized and documented and their limits present in the plants should be estimated. Hence, to avoid the misuse of harmful plant material it is necessary to scientifically develop a pharmacognostical and physicochemical standards of a particular plant material which may ensure and maintain its quality, efficacy and safety profile. The parameters, which are being reported first time in this work, could be useful in the preparation of the herbal monograph for proper identification and authentication of *A. dichotoma*.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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