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PHARMACOGNOSTICAL STANDARDAISATION OF THE FLOWERS OF *QUISQUALIS INDICA*

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ABSTRACT

The present study was undertaken to investigate, morphological anatomical antimicrobial and phytochemical profiling of *Quisqualis indica* flower. Various pharmacognostic parameters evaluated in this study helps in botanical identification and standardization flower of *Q.indica* in crude form and provide the authentic data for the researchers and scientists involved in carrying out further research on this plant part. Results of the histochemical study revealed that flowers of *Q.indica* presence of flavonoids and alkaloids. The phytochemical screening aqueous and methanolic extract of *Q.indica* flower showed that the presence of, steroids, saponins, terepenoids, glycosides, anthroquinone and protein Flavonoids, triterpenoids alkaloids while tannin, alkaloids, protein, carbohydrate, phlobatannins, phenolics were absent. Methanol extract of *Quisqualis indica* flower showed that the presence of alkaloids, steroids, saponins, Flavonoids, triterpenoids, phenolics, nthriquinone and glycosides while tannin, alkaloids, protein, phlobatannins, were absent. The results reveal that extract of *Q.indica* were significantly effective against both bacteria *E.coli*, *St.aureus* and fungi *C.albicans* and *Aspergillus flavus*. The flowers of Quisqualis indica are a newly discovered potential source of natural antimicrobial compounds. The synergistic effect of plant extract against resistant bacteria and fungi leads to new choices for the treatment of infectious diseases. All these data obtained with the present investigation supported the traditional claim associated with *Q.indica* literature.

KEYWORDS: Pharmacognostic, Phytochemicals, Histochemical, Antibacteria.

INTRODUCTION

Nature has been a source of medicinal agent for thousands of years and has an impressive number of modern drugs have been isolated from them, many based on their use in traditional medicine. Medicinal plants have the capacity to produce a large number of pytochemicals with complex structural diversity that is known as secondary metabolites. Some of these secondary metabolites are produced for self-defense. Over the last 20 years, a large number of secondary metabolites from different plant have been evaluated for their antimicrobial, hepatoprotective, anti-inflammatory, and anticancer activity. The demand on plant based therapeutics has increasing in both developing and developed countries due to growing recognition that they are natural products, non nacrotic, easily biodegradable, pose minimum environmental hazards, have no adverse side-effects and are easily available at affordable prices (Anonymou, 1997).

On a global basis, at least 130 drugs, all single chemical entities extracted from higher plant or modified further synthetically are currently in use. A wide range of medicinal plant parts is used for extract s raw drugs and they posses varied medicinal properties. The different parts used include, root, stem, flower, leaves. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated. Systematic investigation was undertaken to screen the antimicrobial activity of selected medicinal plants against oral bacterial infection. To overcome this problem, bioactive compounds with no side effects have to be identified from the medicinal plants. Now a-days herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects. World Health Organization (WHO) estimates that 80% of the population relies on plant based products for human health care (WHO, 1998).

Q.indica belongs to the family of Combretaceae. A plant grows for the first six months as an erect shrub, and then it ends out a runner-from the roots which soon becomes stouter and stronger than the original stem. It is a charming plant. A native a Burma and Malaysian Archipelage, and thrives well in most parts of India, being frequently cultivated in gardens. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification (Kaisar et al., 2009). Pharmacognosy test is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken (Rang et al., 2011). On the basis of the above facts and information, the

present work has been designed and planned to study.

MATERIALS AND METHODS

Collection of plant materials

The while plants of *Q.uisqualis indica* were collected from Kunthavai Naachiyaar College campus, Thanjavur, Tamil Nadu, India.

Pharmacognostical study

Anotomical Studies

The fresh flower, stem and leaves of *Q.indica* were collected and free hand sections were taken to obtain a thin section. The transverse sections of flower was taken on a glass slide to which are added a few drops of chloral hydrate and was heated for 1-2 min, After placing a cover slip, care should be taken to avoid air bubbles and to see that there is sufficient chloral hydrate under the cover slip. Excess of chloral hydrate outside the cover slip is to be withdrawn using a blotting paper (Chloral hydrate is used to clear the tissues and to bring in clarity of the view) Lignified tissue is to be confirmed by staining. To the powder a few drops of mixture of 1:1 Phloroglucinol + Conc HCl was added and after 3 to 4 minutes observed under microscope. The well-known identifying characters were taken Photomicrographs by Sony digital camera under microscope (10 x & 40x) (Wallis, 1989; Dutta, 1971; Trease and Evan, 1984).

Preparation of plant powder

The flowers of *Q.indica* were collected and dried under shade. These dried flowers were mechanically powdered and stored in an airtight container. These powdered materials were used for further analysis.

Physicochemical character

Phytochemical screening

Phytochemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Histochemical analysis

The flowers powder of *Q.indica* were treated with specific chemicals and reagents. The treated powder was further analysed in light microscope. The *Q.indica* treated with

phloroglucinol and diluted HCl gave red color indicates lignin, treated with diluted ammonia and H_2SO_4 gave yellow color indicates flavonoids and treated with Dragant draft reagent gave brown color indicates alkaloids.

Determination of Fluorescence behavior of plant powder

Fluorescence analysis of Phenytoin powder has been carried out in daylight and under U.V light. Florescence analysis of powder of Phenytoin was carried out by the treatment of different chemical reagents such as methanol, H_2SO_4, HCl, HNO_3, NaOH, acetone, hexane, chloroform and distilled water. The powders were observed in normal daylight and under short (245nm) and long U.V. light (365 nm).

Determination of antimicrobial activity

Antibiogram was done by disc diffusion method (NCCLS, 1993) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus* and *Escherichia coli* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans* was spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS

Microscopical characters

T.S of stem

Transverse section of stem reveals outer epidermis, cortex and inner vascular zone. Epidermis in made up of single layered, rectangular shape parenchyma cells, size 7 to 8mm length vanes in diameter. Unicellular trichomes 4x2 to 5x3µwere arise from epidermal cell. Next to the epidermis in hypodermis, made up of 3 layers of compactly arranged, hexagonal parenchyma

cells, 3x2 to 4x2µ in size. Cortex is made up of 7 layers parenchyma cells 3x4µ in size. Sand crystals were found in few cells. Endodermis in single layered distinct, xylem is endarch, meta xylem tube phases outside. Phloem consist of sieve element companion cell and phloem parenchyma. Xylem is transverse by medulary ray and contains axial, ray parenchyma cells. Pith is present made up of parenchymacells. Phenolics and tanniniferous cells were present in the cortex and pith (Fig 1 a).

T.S of petiole

Reveals the following zone; outer epidermis cortex, vascular zone and pith. Epidermis in single layered, cells are rectangular in shape and compactly arranged, multicellular, uniseriate trichomes are found in the epidermis. In the cortex region spherical parenchyma cells are arranged into 4 to 5 layers 3x2 to 4x2µ in size. Endodermis is single layered, vascular zone in differentiated into our 2 to 3 layers of phloem and inner xylem. Xylem is endarch. Pith is small, made up of spherical parenchyma cells arranged with intercellular spaces. Few cells are filled with tannin and phenolic content (Fig 1 b).

T.S of leaf

T.S of leaves of *Q.indica* shows epidermis, mesophyll and vascular region. Upper epidermis is single layer, rectangular shape of parenchyma cells size variables from 3x2 to 5x3µ. Cuticle is absent. In the midrib region few cells in the upper and lower epidermis is smaller in size arranged in horizontally. Next to the epidermis is collenchymatous hypodermis, 2 to 3 layers of collenchyma cells are arranged in both side of the vascular bundle. Vascular bundle is creasant shaped, xylem phases towards upper epidermis and phloem towards lower epidermis. Number of calcium oxalate crystals were observed in the abaxial side of the laminal. In the lamina mesophyll is differenciated in upper palisade and lower spongy parenchyma cell. Palisade cells are elongated cells, filled with chloroplast arranged in two rows. Spongy parenchyma cells are loosely arranged with inter cellular spaces. Stomata are anamocytic (Fig 1 c).

T.S of petal

T.S petal shows outer epidermis, and inner cortical parenchyma cells. Abundance of unicellular trichomes was observed in the upper epidermal region. Number of vascularbundles was present in the petal. Epidermis single layered is followed by 3 to 4 layers of cortex are made up of parenchyma cells, number of mucilage cells were also

observed in the cortex. Numerous, distinct small vascular bundles were present which encloses xylem and phloem (Fig 1).

Histochemical studies

Table 1 reveals the histochemical study of the flowers of *Q.indica* secondary metabolites phenols, saponins, lignin, flavonoids, alkaloids were observed in the powdered petals (Table 1).

Fluorescence behaviour

Behaviors of powder of petals of Q.indica are treated with various chemical reagents and their fluorescent behaviors are given in Table 2.

Powdered analysis

Leaf: Green in color when fresh, powdered leaves are dark green, when drying. Taste bitter, texture is smooth.

Petal: Pink or red in color when fresh, powdered petals are brown, when drying. Taste bitter, texture smooth and fine.

Physicochemical analysis flower of *Q.indica*

Physicochemical parameters such as loss on drying, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive. Alcohol soluble extractive value is 12%, whereas water soluble extractive value is 6%. Table 4.

Qualitative analysis

The phytochemical characters of the flowers of *Q.indica* investigated and summarized in Table 3. The phytochemical screening aqueous extract of *Quisqualis indica* flower shows that the presence of, steroids, saponins, terepenoids, glycosides, anthroquinone, protein Flavonoids, triterpenoids alkaloids while tannin, alkaloids, protein, carbohydrate, phlobatannins, phenolics were absent. Methanol extract of flower *Q.indica* showed that the presence of alkaloids, steroids, saponins, Flavonoids, triterpenoids, phenolics, anthriquinone and glycosides while tannin, alkaloids, protein, phlobatannins, were absent. Qualitative studies reveals the amount of saponins and flavinoids were 0.20 mg/g, terpenoid 30gram and polyphenols were 16.24 mg/g. The amount of polyphenols were high when compared to the others Table 3.

Antimicrobial activity

The *in vitro* antimicrobial activity of the flowers of *Q.indica* against bacteria E.coli stephylococus and fungi *Candida albicans* were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 2. The inhibitory activities in culture media of the *Quisqualis indica* reported in Table 5 were comparable with standard antimicrobiotic viz. chloromphenical and fluconazole. Maximum inhibitory activity was observed in *E. coli* than *S. aures*. Maximum anti-fungal activity was observed in *Candida albicans* when compared to *A. flavinus*.

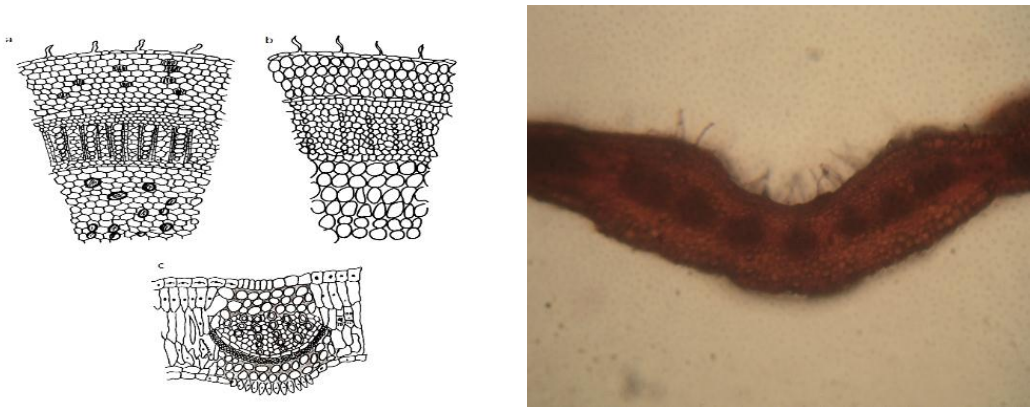

Fig 1: a) Transverse section of the stem of *Q.indica*. b) Transverse section of the petiole of *Q.indica*. c) Transverse section of the leaf of *Q.indica* and transverse section of the flower petal.

Table 1: Histochemical studies of flower of *Q.indica.*

S.No.	Secondary metabolites	Reagents	Observation	*Quisqualis indica* flower
1	Lignin	Phloroglucinol + concentrate Hcl	Red/Pink	+
2	Flavonoids	Dilute Ammonia + H_2SO_4	Yellow	+
3	Alkaloids	Mayers Reagent	Reddish Brown	+
4	Tannin	$Fecl_3$ Solution	Dark Blue to Black	++
5	Crystals	Conc Hcl	Dark Black	+
6	Starch grain	Iodine	Blue	+
7	Steroids	Liberman (5 drops of acetic anhydride + 5 drops of H_2SO_4	Violet to Blue (or) Green	+
8	Poly phenol	Toludine blue	Blue green/Red	+
9	Terpenoids	Dinitrophenol hydrazine (few drops)	Orange	+
10	Saponin	$H_2S\ O_4$ (few drops)	Yellow	+

(+) Presence; (-) Absence

Table 2: Fluorescence behavior of flower *Quisqualis indica.*

S.NO	Test	Visible Light	Short UV	Long UV
1	Powdered Petals	Light Green	Brown	Light Blue
2	Powdered Petals +H_2O	Brown	Green	Blue
3	Powdered Petals+ Hexane	Brown	Light Green	Blue
4	Powdered Petals + Chloroform	Brown	Brown	Blue
5	Powdered Petals + Methanol	Green	Black	Black
6	Powdered Petals + acetone	Pink	Light Black	Blue
7	Powdered Petals + IN Sodium hydroxide in water	Yellow	Green	Black
8	Powdered Petals+ IN Hydrochloric acid	Red	Black	Blue
9	Powdered Petals + sulphuric acid with equal amount of water	Black	Black	Black
10	Powdered Petals + Nitric acid diluted with equal amount of water	Yellow	Light Green	Blue

Table 3: Phytochemical screening of *Quisqualis indica.*

	Secondary Metabolites	Aqueous	70% Methanol	Quantitative Estimation
1	Tannin	++	+	-
2	Phlobatannins	+	-	-
3	Saponin	+	+	0.20 (mg/g)
4	Flavonoids	+	+	0.20(mg/g)
5	Steroids	+	-	-
6	Terpenoids	+	+	0.30(mg/g)
7	Tritrpenoids	+	+	-
8	Alkaloids	+	-	-
9	Carbohydrate	+	+	-
10	Protein	+	+	-
11	Anthroquinone	+	+	-
12	Polyphenol	+	+	16.24(mg/g)
13	Glycoside	+	+	-

(+) Presence (-) Absence

Table 4: Physicochemical parameters of flower *Q.indica.*

S.No	Tests	As Per Analysis
1	Description	Pale green colored fine powder
2	Moisture content	1.10%
3	Ash value	0.140%
4	Acid insoluble	0.70%
5	Water soluble	6.0%
6	Alcohol soluble	12.0/%

Table: 5 Antimicrobial activities of flower of *Quisqualis indica.*

zone of inhibition in diameter (mm)				
Microbial Organism	50µl	100 µl	150 µl	Standard
Escherichia coli (mm)	4.50±0.31	5.00±0.35	6.50±0.45	8.00±0.56
Staphylococcus aureus (mm)	3.50±0.24	5.00±0.35	5.20±0.36	8.70±0.60
Candida albicans (mm)	6.20±0.43	7.00±0.49	7.20±0.50	8.20±0.57
Aspergillu flavus (mm)	5.50±0.38	5.70±0.39	6.50±0.45	7.20±0.50

Values were expressed as Mean ± SD. Bacterial standard – Chloromphenical;

Fungal standard - Fluconazole.

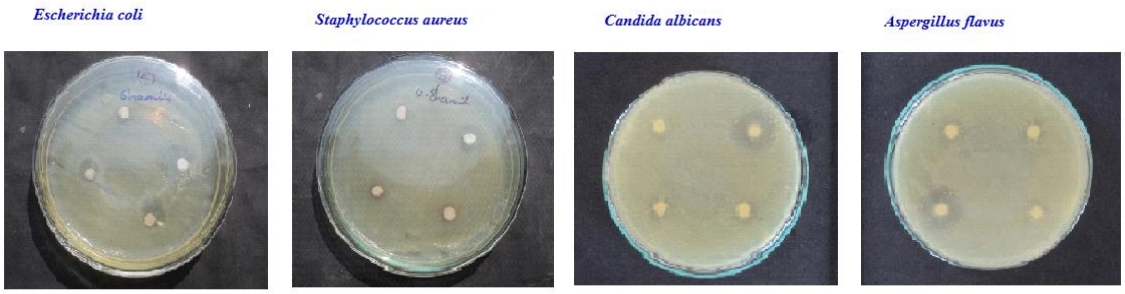

Fig: 2 Antimicrobial activities of *Quisqualis indica.*

DISCUSSION

Pharmacognosy may be defined as "an applied science that deals with the biologic, biochemical and economic feature of natural drugs and their constituents." Modern aspects of science include not only the crude drugs but also their natural derivatives. Plant anatomy, in turn, has given rise to the independent science of cytology, which is the study of the cell, a rapidly developing field that plays a great role in the understanding of vital processes in general and of the phenomena of heredity and mutability in particular. Plant Anatomy is the branch of botany concerned with the internal structure of plants. It is closely related to plant physiology, the science of the vital processes which take place in plants.

Anatomical characters of the present study reveals the semi xerophytic nature of the plant. Mechanical tissues cuticle were not observed in the petiole, stem and leaves. The leaves contain two layers of palisade cells, it is an identifying character *Q.indica*. Anatomical studies of flowers shows mucilage cells in the cortex. Mucilage cells and flavonoid in the petals may be responsible for the fragrance of the flower. According to WHO (1996, 1982 and 1993) standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and

product promotion. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The standardization of crude drugs is important before any work carried out.

Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron (Krishnan, 2001). The importance of histochemistry in solving critical biosystematics problems is as popular as the use of other markers. Represents histochemical studies of flowers of *Q.indica*. This study further confirmed the presence of phytochemicals in *Q.indica.*

Fluorescence behavior of flower was investigated by addition of acid or alkali. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many products, which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some drugs are often assessed qualitatively in this way and it is an important parameter of potential use as an imaging agent. Table 4 represents Fluorescence behavior of flower powder.

The quality control methods play on important role in traditional medicine which conserve as a tool for identification, authentication and quality control of herbal drugs. Physicochemical analysis reveals. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of Bacteria, Yeast or Fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not.

Raghavendra, (2006) examined the powdered flower material of different solvent of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Extracted eight bioactive compounds from dry flower of *Cnidoscolus aconitifolius* using water and ethanol. Different extracts of *Semecarpus anacardium* were analyses for its phytochemical properties.

Present study reveals the flowers of *Q.indica* contains alkaloids, phenols, tannins, flavonoids, saponins. Flavonoids have shown potential antioxidant and anti-inflammatory activities. Tannins have a study astringent action and are reported to have antibacterial and anti-inflammatory activities (Schulz *et al.,* 2002). This result is similar to that of our study. Presence of high phenolic compounds in the flowers of *Q.indica* can actors a good source of natural medicine. Earlier reports of extract of *Q.indica* was screened against Escherichia coli and Staphylococcus aureus species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. Earlier reports of *Q.indica* Showing various pharmacological activities such as anti-inflammatory activity, antipyretic activity, immunomodulatory activity, anti-staphylococcal activity. Anthelmintic activity, antiseptic activity etc due to its presence of various active constituents all over the parts of plants. This plant contains some medicinally active phytochemical constituents which are responsible for various pharmacological activities.

CONCLUSION

Various pharmacognostic parameters evaluated in this study helps in botanical identification and standardization flower of *Q.indica* even in crude form and provide the authentic data for the researchers and scientists involved in carrying out further research on this plant part. The most important bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds. These are the important raw materials for drug production. Most plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganism. Overall, the *Quisqualis indica* flower are a rich source of phytochemicals and potential antimicrobial activity that can be important in infectious disease prevention.

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