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Histochemical exploration and phytotherapeutic evaluation of antimicrobial properties of two selected exotic fruit trees and legume known in Tamil folk medicine

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Abstract
The current resurgence in crude drug research and its moorings in underpinning the importance of phytochemicals provide the cue to study plants implicated as remedies in traditional heath care assists. The two exotic trees *Adansonia digitata* and *Crescentia cujete* and the medicinal seed legume *Caesalpinia bonduc* known for long in regional and local traditions investigated here show the species to contain constituents such as glycosides, flavonoids, phytosterols, tannins, free aminoacids, fixed oils and fats in somatic tissues, fruit pulp and seeds which can possibly be used as medicaments. As preliminary report on histochemical aspects alongside observations made on the feasibility quelling and controlling certain common potential pathogens lends credence to age old belief and claim that the said species can be hired in an organ specific and case specific manner to treat ailments. This fortifies the significance of TM in dealing with infections and treating vexing health disorders.

Keywords: TM –Traditional Medicine; Histochemical Stains; Antimicrobial Assays; Crude Drug; Phytotherapeutic

INTRODUCTION

Like any other proven traditional system of medicine, Siddha the native Tamil medicine backed by classical records (Murugesa Muthaliar, 1936; Kannusamy Pillai, 1939) and newer compilations (Karunamoorthi *et al.*, 2012; Rana *et al.*, 2021), although the word of mouth secrets exchanged within the clan still requires verification and validation (Subbarayappa, 1997; Sen *et al.*, 2017). It is interesting to note that besides indigenous plants, trees from offshore for non-medical purposes have also been recognized by locals to cure many common and rare aliments (Shanmugam *et al.*, 2020). Owing to their arborescence and perennial nature, it can be expected that tree species harbor many phytochemcials and produce huge fruits and seeds with a host of secondary metabolites to find use as food, health supplements and as ethnic medicine (Khan, 2015). This study aims to probe the therapeutic potential of two such exotic trees, the Baobab (*Adansonia digitata* L.) and Calabash (*Cresenctia cujete* L.), in comparison with a popular large seeded leguminous medicinal straggler *Caesalpina bonduc* Roxb.

Adansoina digitata L., locally referred as *Pappara puli* or *Annai puli* (Tamil) owing to the fruit, gets referred as source of therapeutic in the popular Siddha treatise *Gunapadam* and is known to be a useful astringent, diuretic and demulcent. Recognizing the genus under Bombacaceae, it has been pointed out that the fruit pulp can be administered as an antipyretic and can be helpful in controlling biliousness, vomiting, dysentery and excessive perspiration. Though the significance attached is variable from place to place, the taxon is largely accepted to be a cure for bilious dyspepsia, toothache, gingivitis, putrid pestilential fever and is used as a emmenagogue, febrifuge, emollient, diaphoretic, and a famine

food besides being a prophylactic and a protectent (Ismail et al., 2019). As a tall, massive and robust deciduous perennial, Baobab known for its gait, large buttress and aesthetically appealing foliage is considered an important introduction in India from Africa. Baobab is seen both in wild and domestication in India for long and therefore gets referred in regional medicinal literature (Mishra *et al*., 2019).

Tree Calabash (*Crescentia cujete* L., family Bignoniaceae), in contrast is a moderately sized tree from West Indies and Guyana brought to India from tropical America. Seen planted in India mostly in gardens is groomed and nurtured for its Caulioflory, the tree serves as beautiful ornamental with purple to pink stalked lowers and florets on the trunk. It is found with fruits year round and the large green, ovoid, fruits (30 cm across) bound with hard sclerified shell popularly called *Thiruvodu* gains its prominence for its aesthetic appeal. Mesocarp of ripe fruit is made into a poultice and applied topically for headache and the leaves of *C. cujete* are used as emetic, purgative diaphoretic and can be used to treat bruised wounds, inflamed joints, sprains, ear aches and other ear complaints. The sweet-sour fleshy pulp if isolated and used fresh is reported to be a diuretic, antipyretic, aperient, febrifuge and a cure for urinary problems, diabetes mellitus and even heart ailments besides its antibacterial activity and allelopathic effects (Ejelonu *et al.*, 2011; Mackenzie Theis *et al*., 2017).

Unlike the aforementioned taxa with non-conventional fleshy fruits, the prickly, armed legume Bonduc nut or Physic nut, *Caesalpinia bonduc* Roxb is straggler that forms seeds in its semi-dry pods. Familiar to traditional medicinal practitioners *Kazharchikai* is used both externally and internally to treat a number of ailments including infertility and can be used as antiperiodic, antispasmodic, tonic, febrifuge and anthelmintic (Singh and Raghav, 2012; Gadakh *et al*., 2020). The large spherical seeds known to rural folks for its therapeutic role is reported to be a bitter, acrid, astringent with thermogenic properties used as anodyne, anti-inflammatory, digestive, stomachic, depurative, expectorant and anti microbial rather than its edibility (Sembiring, et al., 2018). Reports of being used as a contraceptive, antipyretic, anti-diabetic, rabifacient, besides providing for a liver tonic and a cure for hydrocele for make the species as a much sought after medicinal.

Past studies undertaken on *Adansoina digitata, Crescentia cujete* and *Ceasalpinnia bonduc* have focused either on their broader utility or in isolated understanding over the medicinal efficacy of these plants. Research pursued on the leaves and stem have provided clues that ingredients such as oils, phenols, flavones, alkaloids and glycoside contained in these plants could elicit antibacterial properties (Cushine *et al*., 2014; Sarubu *et al.*, 2019). Although fruits and seeds of these plants are used in community medicine only limited studies have been taken up on organ specific effects that there is a paucity of dataon histochemical aspects. In view of the growing interests in naturopathy with an assurance to negate adulterants in the context of resource-constrained health care settings screening phytochemical constituents through histochemical staining and preliminary phytochemical tests becomes a necessity.

MATERIALS AND METHODS

1 Plant Material

Selected trees *Adansonia digitata* L. and *Crescentia cujete* L. and the straggling legume, *Caesalpinnia bonduc* Roxb., growing in Madurai and its suburbs offered the material for this study. Collections of the fruits and seeds, chosen plants identified by the resident taxonomist who could authenticate every collection confirmed by comparison with voucher specimen (No. AMC /HCN//28, 29, 30). As fresh samples were used for anatomical investigations, crude plant extracts of leaves, fruit and seed that was prepared after shade drying following due precautions. Thin handmade sections colored appropriately with stains and reagents helped *in situ* histochemical localization of various plant constituents. Shade dried samples were pulverized and for solvent extraction both cold maceration and/or hot percolation as well.

2 Preparation of Crude Extracts

Leaf samples and the pulp from the indehiscent fruits of the trees, and the mature seeds of the legume *C. bonduc* were allowed completely to dry and loose moisture under shade and the resultant dry samples were macerated in a mixer grinder. Sieving away the fibers and particulate substances using a #40 mesh, the sifted as per the procedure described in a previous paper (Mohan et al., 2020). Air dried coarse plant powder, 1.3 kg in weight in each case was submitted to sequential maceration with 3500 ml of petroleum ether, chloroform, and methanol one after the other at the temperature based on the

order of polarity, After each step the filtrate is collected and reside(marc) is carried forward elution with the next solvent. Crude extract collected was evaporated to dryness under reduced pressure in Eyele Rotary evaporator (Japan) and on determining and recording the yield; the residue was dissolved with the universal solvent DMSO and used in preliminary phytochemical tests and anti-microbial assays.

3 Preliminary Phytochemical Tests

Identification of the various phytochemical constituents was carried out adopting the appropriate procedures outlined by Trease and Evans (1997).

Test for Phytosterols

Two different tests namely (i) *Salkowski Test* with the sampled extracts dissolved in chloroform and treated equal volume of concentrated sulphuric acid to form a bluish red to cherry red color as positive result, and (ii) *Libermann-Burchard's Test* for sterols involving 50 mg of extract dissolved in 2ml of acetic anhydride added with a drop or two of concentrated sulphuric acid slided along the slant test tube to form a red ring at the interface of two liquids, helped checking out the presence of phytosterols.

Test for Alkaloids

Qualitative tests for alkaloids were pursued using Mayer's reagent with mercuric chloride and potassium iodide offering a yellowish buff colored precipitate, Dragendorff's reagent resulting the orange-brown precipitate and Wagner's reagent yielding reddish brown precipitate respectively were expected as positive responses for alkaloids.

Test for Glycosides

A combo of testing procedures hiring Libermann-Burchard's test offering to dynamic color change response and Legal's test involving pyridine and sodium nitroprusside solution and Borndrager's test showing up in pink colour revealing anthroquinone glycosides were used for spotting the occurrence of glycosides.

Test for Flavonoids

Plant extract eluted with solvents subjected to Ferric chloride test imparting a blackish red and Shinoda's test with magenta colour provided the indications for the presence of flavonoids.

Test for Tannins

Tannin one of most common ingredient in all three sample sources was noticed by the development of yellowish brown precipitate with 10% aqueous Potassium dichromate solution, 10% aqueous lead acetate solution and formation of greenish black coloration in response to 1ml of 5% Ferric chloride solution.

Test for Phenolic Compounds

The filtrate of extracts treated with 5% w/v ferric chloride solution forming a black precipitate, and lead acetate solution so as to form white precipitate offered the positive result presenting the likelihood of the occurrence of phenolic compounds.

Test for Saponins

Small quantities of solvent free extract collected after cold maceration dissolved in minimum amount of distilled water and shaken vigorously in graduated cylinder for 15 minutes formed foam indicating the presence of Saponins.

Test for Reducing Sugar and Amino Acids

Molish's test, Benedict's reagent and Fehling's solution were mixed in a test tube with extracts were tired to test carbohydrates and small quantities of the extract treated with Ninhydrin (Triketohydrindene hydrate) at the pH range of 4 to 8 offered to the recognition of amino acids.

Test for Gums and Mucilage

Small quantities of the solvent free samples treated separately with absolute alcohol, stirred and filtered can be tested for gums targeting the swelling property.

4. Histochemical Staining

Anatomical investigations aimed at understanding the organization of tissues and histochemical studies probing with an intent of localizing biochemical specific metabolites were pressed in with the stem, petiole and leaf sections of all three plants. Fresh free hand transverse sections subjected to case specific staining probed with Carl Zeiss phase contrast microscope under varying magnification provided on the possible presence of starch, oils, lipids, phenolics, alkaloids,

terpenoids, phytosterols, tannins and other specialized structures. Stains such as Safranin, Fast green, Coomassie brilliant blue, Tannic acid, I_2KI, Sudan IV, Dragendorff reagent, Ferric Chloride and Vanillin were used as per techniques described earlier (Krishnamurthy, 1988).

Safranin meant for staining lignocellulosic components of plant cell wall, Fast green to identify the general proteins, Coomassie Brilliant Blue R 250 prepared by dissolving 0.02 g of powder in 100 ml of 1% acetic acid to locate proteins blue spots, Tannic acid with the tendency to precipitate proteins to exert clearing response, Iodine potassium iodide (I_2KI) to stain starch dark blue to black in colour, and Sudan IV was used to identify the lipids served in the detection of the common metabolites. In the screening for secondary metabolites, Dragendorff reagent locating alkaloids with orange brown color, Ferric chloride test staining tannins as blue green precipitates, and Vanillin meant for staining terpenoids pink were used.

***5.* Antimicrobial Assay**

Stock cultures of four different microorganisms namely *Escherichia coli* (MTCC 406), *Klebsiella pneumonia* (MTCC 4031), *Pseudomonas aeruginosa* (MTCC 1688) and *Staphylococcus aureus* (MTCC 96) procured from Microbial Type Culture Collection centre (IMTECH) culture collections were used as test organisms. On ascertaining the viability at 0.5 McFarland standard (approximate cell density $1.8x10^8$ CFU/ml with an absorbance of 0.132 at wavelength of 600nm), active cultures were raised and maintained separately. As active inoculums were maintained by daily subcultures, Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the test samples of crude extracts were ascertained by micro and macro dilution broth technique.

With the standard procedures followed in the preparation Muller Hinton medium (Hi media Mumbai) preparation, a total 96 different test samples (four solvent inclusive of an aqueous preparation each representing the leaf and seed/ fruit pulp samples of all three plants) were assessed well diffusion techniques adopting the method of assay described by (Holder and Boyce, 1994). Results gathered from experiments preformed in triplicates and repeated six times targeting specific pharmacological action were evaluated appropriately and are expressed as a mean of not less than 6 values in all experiments.

RESULTS

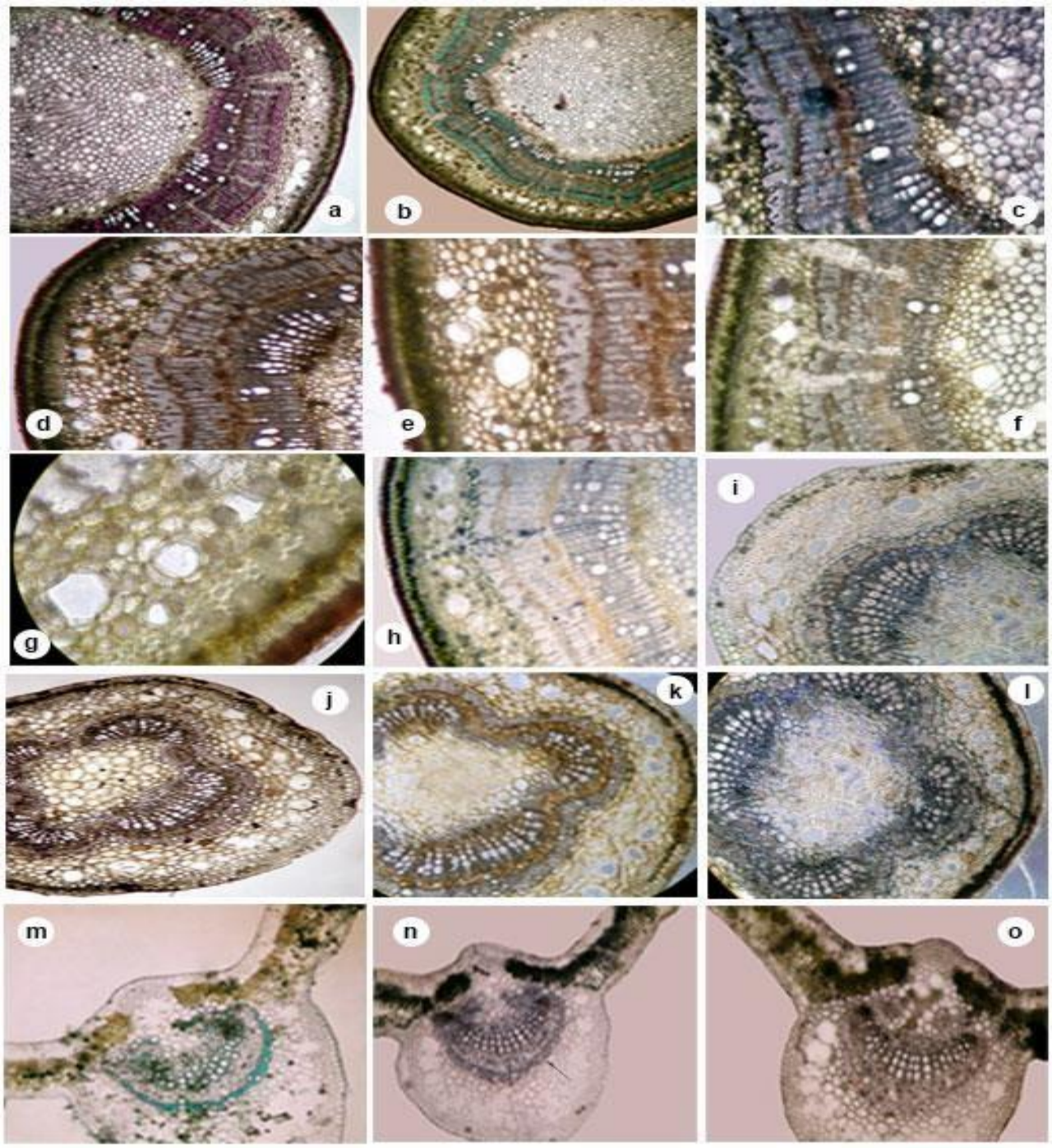
I Screening of phytochemical constituents

Microscopic methods involving histochemical and phytochemical procedures helped probing phytochemical present in the selected three species. Thin hand sections of leaf, stem and petiole of *Adansonia digitata*, *Crescentia cujete*, and *Caesalpinia bonduc* treated with suitable stains offered to the localized detection of chemical substances in somatic tissues while the solvent extracts eluted from fruits pulp and seeds tested with preliminary phytochemical tests provided for qualitative assessment of the therapeutic milieu.

Studies on Adansonia digitata

Juvenile cylindrical shoots that emerged in flushes of the defoliated tall tree with a typical massive buttressed trunk served as the material for histochemical examination (Table-1). Free hand sections of the twigs stained individually with safranin and fast green showed the anatomical organization of the young stem in a rejuvenating branch (Plate-1a, b). Sections made with the stem showed a well-defined epidermis, greenish parenchymatous cortex and the prominent concentric rows of protein rich layers of protophloem fibres in vascular region stained with cooomasie blue (Plate-1c) besides the central parenchymatouspith offers clues to substantial presence of chlorophyll, oil globules and the primary metabolites as well. As stem sections stained with tannic acid (Plate-3d) and sudan IV (Plate-1e) showed the basic histological organization and detected the presence of lipids at the epidermal surface, those that treated with ferric chloride showed higher tannin content in emerald green shade (Plate-1f). It could deduced from plate 1g that a crystalline inclusions and cavities and are common in thecortical regions, while cells in the outer pith spotted in orange tone indicating the occurrence of alkaloids (Plate-1h).

Plate-1 Handmade sections of stem, petiole and leaf of *Adansonia digitata*



Cross sections. of Baobab stem stained with (a) saffranin, (b) fast green, (c) coomasie Brilliant Blue, (d) I_2KI, (e) sudan IV, (f) ferric chloride, with a closer view of (g) cortex revealing cell inclusions and (h) alkaloids containing cells at pith with Dragendorff reagent. Petiole segments colored with (i) coomasie blue, (j) tannic acid is contrasted from leaf anatomy discerned through (m) fast green, (n) I_2KI and (o) ferricchloide staining.

Cross section of the petiole measured about 0.5 to 1 cm in diameter nearly reflecting a similar histological constitution of the stem which also revealing a definite indication of coomasie blue staining protein rich sub-endodermal tissues (Plate-1i), highlighted with coagulated proteins in the protophloem fibers with Tannic acid (Plate-1j) clearly highlighted anatomical variations and the histochemical differences. Cells eliciting orange brown alkaloid containing cells proximal to endodermis in response Dragendorff (Plate-1k) could be contrasted from the bluish black starch containing sub epidermal layers in section floated in I_2KI (Plate-1l) eliciting finer changes in the cytochemistry of constituent cells and tissues in the genus. Cells of cortex turned emerald green in color as response of ferric chloride reaction to tannin quite liberally. It may be noticed that the vasculature is traversed by metatracheal elements and 4 to 10 cells wide conspicuous rays interspacing vasculature is at proximity to the orange brown cell layer suggesting the availability of alkaloids. Leaf sections vouched histochemical diversity of the Boabab, with a prominent cytosolic protein rich bundle sheath (Plate-1m), starch rich palisade (Plate-1n) and profuse but scattered tannin presence at the leaf laminar regions (Plate-1o) respectively.

TABLE-1
COMPARATIVE DATA ON STAINING OF FREE HAND SECTION

Stains Metabolites	*Adansonia digitata*			*Crescentia cujete*			*Ceasalpinia bonduc*		
Plant organ	Stem	Petiole	Leaf	Stem	Petiole	Leaf	Stem	Petiole	Leaf
Safranin	+	+	+	+	+	+	+	+	+f
Fast Green	+	+	+	+	+	+	+	+	+f
Coomasie brilliant blue	+	+	+	-	-	-	+	+	+++
I_2 KI	++	++	++	-	+	+++	+	+	+++
Sudan IV	++	+	+f	-	-n	-	-	-	-
Tannic acid	+	+	+	-	-	-	+	+	++
Ferric chloride	++	++	+	+	+	+	+	+	-
Dragendroff	+	+	-	-	++	++	-	+f	+f
Vanillin	-	-	-	+	+	+	-	-	-

- Absent; + mild coloration; (+ f faint); ++ Bright; +++ intense; n- stained in the notch only

Petiole of Baobab tree exhibited a characteristic anatomy with a wedged vascular bundle with lignocellulosic compounds stained with safranin and fast green with the mid portions of the petiole made up of loosely arranged large parenchyma, looking like aerenchyma four principal endarch bundles alongside copious amounts of phloem flanking the cambial tissue surrounded by a less conspicuous crushed bundle sheath. Staining responses with the with coomassie blue highlighting the presence of protein amidst the crystalline inclusions in cortext, tannin containing located by ferric chloride in emerald green color, lipoid epidermal layer and the darkly stained starch containing cells in the periphery of the outer cortex vouch the cytochemical profuseness of the taxon.

Alkaloid containing cells could be observed conspicuously in comparison with only a faint occurrence of terpenoids while the dorsi-ventral leaves reveal thin palisade cell layer backed by substantial spongy mesophyll at the leaf lamina containing chlorophyll except near the central vein where cells are broken down to form a large lacuna for the storing mucilage. Efforts taken to study ingredients of fruits revealed that pulp that stand the severity of drying during the summer revealed the hygroscopic gummy content that could rehydrate with water and could provide for crude extracts with clear positive responses anthraquinone glycosides, flavovnoids, tannins (Table-2). Since the excessively thick sclerified seed coat that wraps up embryos posed problems in grinding, with the non- inclusion of the seed borne ingredients, solvent extracts short of being referred as mesocarp were considered merely as fruit pulp samples.

TABLE-2

QUALITATIVE PHYTOCHEMICAL EXAMINATION WITH SOVLENT EXTRACTS OF SELECTED PLANTS

S.No	Plant constituents tested & Reagent used	*Fruit pulp /Mesocarp*						*Seed extracts of Ceasalpinia bonduc*		
		Adansonia digitata			*Crescentia cujete*					
		PE	CF	ME	PE	CF	ME	PE	CF	ME
1	**TEST FOR ALKALOIDS**									
1.1	Mayer's test	-	-	-	-	-	-	-	-	-
1.2	Dragendorff's test	-	-	-	-	-	-	-	-	-
1.3	Wagner's test	-	-	-	-	-	-	-	-	-
2	**TESTS FOR GLYCOSIDES** (Anthroquinone glycosides)									
2.1	Borntrager's test	-	-	+	-	-	+	-	-	
2.2	Baljet test	-	-	+	-	-	+	-	-	
2.3	Legal's test	-	-	+	-	-	+	-	-	
3	**CARBOHYDRATES**									
3.1	Molish's test	-	-	-	-	-	-	-	-	-
3.2	Fehling solution	-	-	-	-	-	-	-	-	-
3.3	Bendicts reagent	-	-	-	-	-	-	-	-	-
4	**PHYTOSTREOLS**									
4.1	Libermann's and Burchard's	-	-	-	+	+	-	+	+	+
4.2	Salkowskis' test	-	-	-	+	+	-	+	+	+
5	**FLAVONOIDS**									
5.1	Ferric chloride test	-	-	+	-	-	+	-	-	-
5.2	Shinod's test	-	-	+	-	-	+	-	-	-
6	**FIXED OILS AND FATS**									
6.1	Spot test	-	-	-	+	+	+	+	+	+
6.2	Saponification	-	-	-	+	+	+	+	+	+
7	**FREE AMINO ACIDS**									
7.1	Million's reagent	-	-	-	-	-	-	+	+	+
7.2	Ninhydrin reagent	-	-	-	-	-	-	+	+	+
8	**TANNINS**									
8.1	5% Ferric chloride	-	-	+	-	-	+	-	-	-
8.2	10% Lead acetate	-	-	+	-	-	+	-	-	-
9	**SAPONINS(Foam test)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
10	**GUMS AND MUCILAGE**	**-**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**-**

PE- Petroleum Ether; CF-Chloroform; ME-methanol Extract

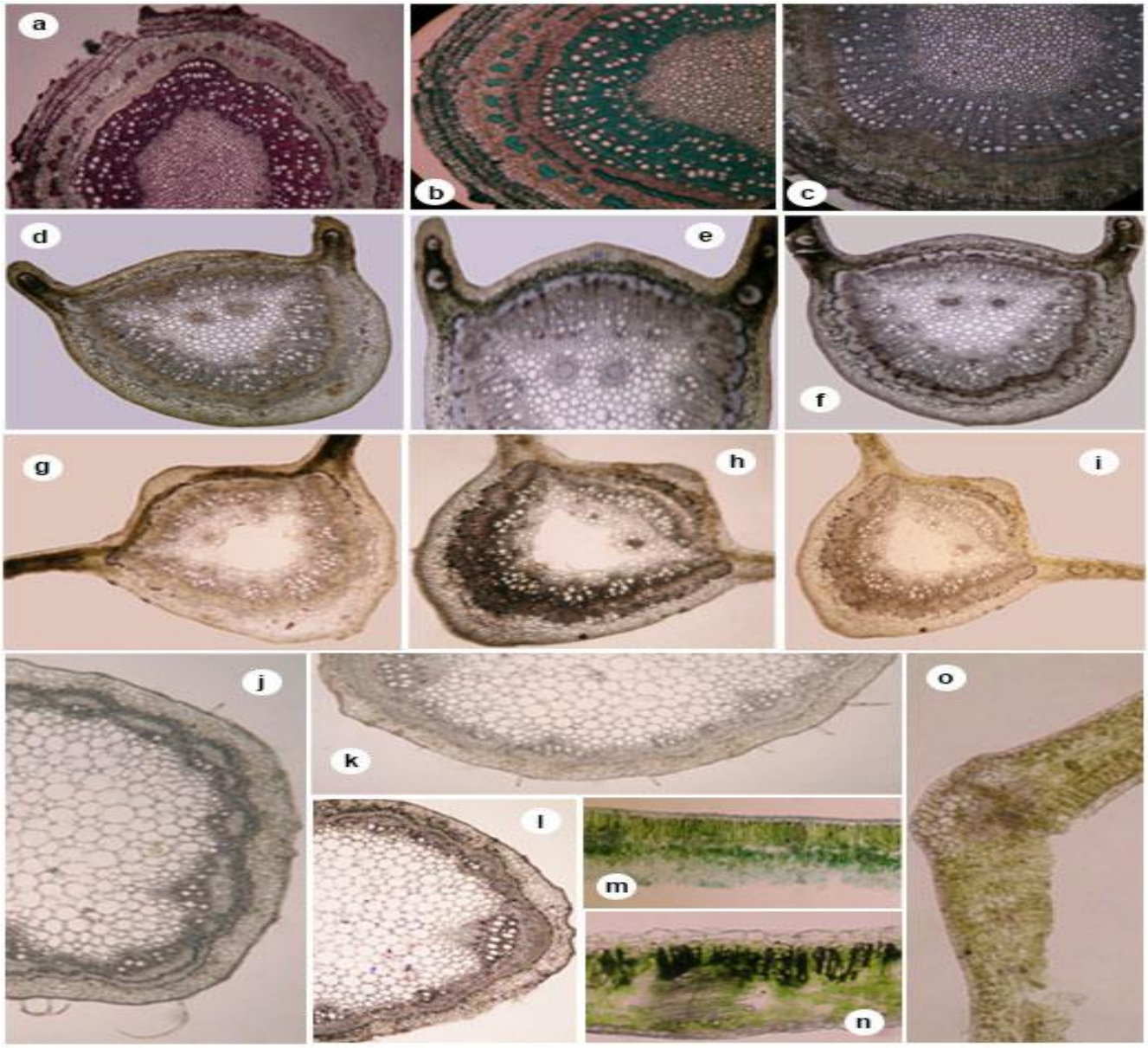
'+'indicates the positive result; '–'indicates the negative result.

Investigations on Crescentia cujete

The cauliflorous *Crescentia cujete* L., bearing clusters of non-deciduous leaves and stalked flowers borne directly on the fissured bark surface is endowed with a physio-morphic state that allowed the moderately sized tree to remain elegant and ever green round the year. Stem portions stained with safranin show fissured lingocellulosic components leaving the discontinuous epidermal system with subjacent outer cortex exhibiting islets of cells with high affinity for fast green amidst the non-staining parenchymatous tissue (Plate-2a, b). Compactly packed xylem with regular prominent vessels forms sound vasculature in which a good amount of phloem is bound by a less conspicuous endodermis. Sections stained with Coomassie blue showed pockets of cell clumps containing proteins and these regions are seen cleared after protein coagulation by tannic acid. Staining attempts with Sudan IV elicited a poor response against the ferric chloride which imparted the emerald green color to a series of cell layers around epidermis and endodermis owing to the rich presence of tannin (Plate-2c). A study of stem andpetiole sections with Orange brown cells with Dragendorff allowed a fair speculation on the presence of alkaloid. Conversely the emerald green shade revealed the presence of tannins in response to ferric chloideand the pink tinge upon the adding vanillin prompted the presence of terpenoids.

Petiole segments excised from the shoots offered an interesting material for study. The long groove on the upper surface of petiole could be inferred as two adaxial ridges in cross sections. Unlike in *Adansonia*, the organization of the stem and petiole were similar with only an additional inclusion of two small central bundles in mid pith. The profuse presence of the cell wall material and rich cytosol content detected in safranin and fast green respectively did not gel well with coomassie brilliant blue staining. It appears that petiole regions are hard-pressed for protein rich cells even though starch deposition is noticed in specific layers beneath the epidermis. Sudan IV highlighted the presence of lipid only at the flapped notch as the orange brown coloration to Dragendorff reagent shows the possibility of alkaloids next to the endodermis (Plate-2d). The fair distribution of tannin can be seen as response to $FeCl_2$ (Plate-2e) and affinity of terpenoids for vanillin can be seen in pink shade at the region between endodermis and he vascular cylinder (Plate-2f).

Plate-2 Stem, petiole and leaf section of *Crescentia cujete* and *Caesalpinia bonduc*



Stem sections of Calabash (a-c) stained with (a) saffranin, (b) fast green, (c) $FeCl_2$, compared with petiole segments stained by (d) Dragendorff reagent, (e) $FeCl_2$ and(f) Vannilin. One can observe leaf colored by Dragendroff reagent I_2KI and Vanillinto indicate alkaloid, tannin and terpenoids at different regions. Micro preparations of Bonduc Nut stem (k to l) stained respectively with I_2KI, Fe cl2 and Dragendroff reagent compared with leaf sections stained with (m) coomasie blue, (n) I_2KI and (j) tannic acid is contrasted from leaf anatomy discerned through (m) fast green, (n) I_2KI and (o) Dragendorff reagent targeting proteins, starch and alkaloids respectively.

Although solvent extracts of the mesocarp (fruit pulp with seeds) of *Crescentia* showed negative response to tests for glycosides, alkaloids and flavonoids, there were clear positive indications for the presence of alkaloid, starch and terpenoids in somatic organs. Cross sections of leaves of this tree stained with Dragendroff reagent I_2KI and Vanillin for these metabolites endorses this view (Plate-2g,h&i). Libermann-Burchard test and Salkowski's test for phytosterol presented an interesting situation. It was found that the petroleum ether and chloroform extracts from seed embryos were positive whereas the methanol fractions collected from the same source was negative. Scanty presence of phenols and saponins can be detected in methanol extracts from fruit pulp. As in pulp samples from *Adansonia*, mesocarp extracts as well as the sections made form somatic organs showed a notable presence of tannins but comparable apolar fractions from seed source evaded detection of gums and mucilage. Instead *Crescentia* is found to rich fixed oils and fats.

Observations on Caesalpinia bonduc

The armed straggler *C. bonduc* that bears a slender, compact, prickly stem with much mature parenchymatous pith presents greater chances for the accumulation of plant metabolites. The cortex region is almost entirely made up of parenchyma rich in cytosol stained with fast green and relatively less lignocellulose as inferred from staining by safranin. Sections stained with coomassie brilliant blue showed the presence of proteins, endorsed by the protein clumping by Tannic acid. When I_2KI was used, the cells adjoining the endodermal layer preferentially took up bluish black color indicating starch (Plate-2j), sections treated with Sudan IV turned red color indicating the presence of lipids in the outer epidermis. Though all of cortex failed to exhibit the emerald green color, a negative response to ferric chloride indicating that tannin is absent (Plate-2k), the orange color imparted by Dragendorff reagent revealed the feasibility of the presence of alkaloids (Plate-2l). Despite doubtful indication of terpenoids by Vanillin, the presence of free amino acids, fatty acids and fixed oils appeared with confirmed certainty.

The thin and delicate leaf of *C.bonduc* lacking prominent veins was a difficult material for study. Bound by a thin epidermis atop, the single row of prominent palisade occupying nearly half the constituent space contained intensely green chlorophyllous tissue masked the staining reactions. Sections stained with coomassie blue showed that the dye gets lodged in the upper epidermis and the spongy parenchyma suggesting copious presence of proteins (Plate-2m). In contrast sections stained with Sudan IV showed positive responses for lipids only at the lower epidermal layer and the palisade found beneath turned intensely dark with I_2KI hinting accumulation of starch (Plate-2n). Studies aimed at locating secondary metabolites were disappointing with ferric chloride and vanillin, and tannin is absent in leaf. If the leaf samples bleached in boiling ethanol for a few seconds, Dragendorff reagent helped detecting alkaloids to some extent (Plate-2o). Seed embryo of *Caesalpinia bonduc* lacking gums and mucilage, tested positive for fixed oils and free amino acids.

II Anti-microbial Assays

Well diffusion technique attempted with bacterial isolates helped in ascertaining the effectiveness of plant extracts. Initial studies focusing on establishing the viability of stock cultures helped in ascertaining the identity of the bacterial species. Among the four species, *E.coli* grew well in MacConkey Agar, *Klebsiella* and *Pseudomonas* grew well on the high chrome UT agar and Muller-Hinton Agar. Isolation and establishment of cultures with *Staphylococcus aureus* was relatively easier Mannitol salt agar medium. With the procedure of gram staining providing for basic characterization, all test organisms maintained and stabilized for its growth in nutrient broth and Muller-Hinton Agar for conducting anti-microbial assay. Streaking inoculums for axenic cultures, plates were prepared to determine anti-bacterial activity through the well diffusion study. The zone of inhibition taken as the reference of antimicrobial activity of the extract, tried at a uniform dosage of sample loaded in 6 mm well, in all species irrespective of the source helped comparing the efficacy of the extracts.

Botanicals differ to offer a specie specific control

Studies pertaining to antimicrobial assays revealed organ specific and species specific variations in results. As it is revealed in Table-3, *Adansonia digitata* catered to significant control in general. It curbed the growth of *E. coli* and *Pseudomonas* on Muller-Hinton agar. With a reasonable control of *Klebsiella* attributed especially to extracts *Crescenta cujete*, the two tree members' elicited a better control on the gram negative forms. When compared with extracts of fruit pulp origin, seed embyro extracts of *Ceasalpinia bonduc* fetched good observations on gram positive *Staphylococcus.* Since the said microbe evaded plant extracts it appears that the gram positive bacterium is quite recalcitrant.

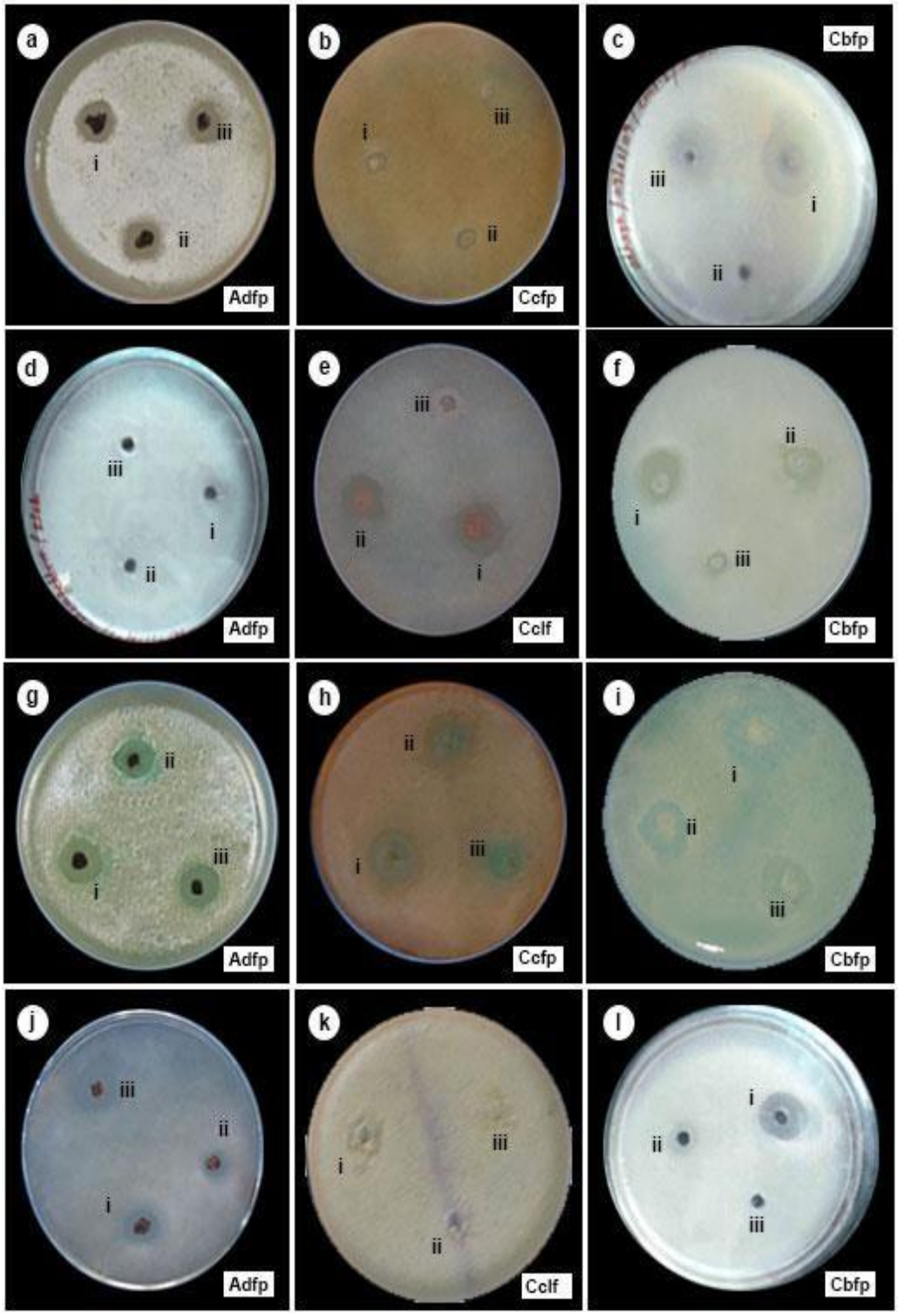
Table: 3 ANTI-MICROBIAL ASSAYS: Activity of different crude plant extracts

Extract code	Tested microbe															
	Escherichia coli				***Klebsiella pneumonia***				***Pseudomonas aeruginosa***				***Staphylococcus aureus***			
	AQ	ME	CF	PE	AQ	ME	CF	PE	AQ	ME	CF	PE	AQ	ME	CF	PE
FP*Ad*	19.6	29.8	27.7	27.8	9.8	16.3	12.5	8.97	30.6	34.3	32.8	30.9	10.5	*15.9*	*18.0*	14.3
L *Ad*	18.0	33.3	26.8	24.1	12.6	15.9	16.1	11.7	26.7	31.4	30.5	27.1	10.1	14.3	15.6	10.7
FP*Cc*	15.3	24.7	21.9	16.0	14.6	28.7	20.8	16.9	12.4	31.8	16.7	14.3	15.6	17.3	17.0	14.5
L*Cc*	17.4	32.1	21.6	20.8	16.0	24.8	22.9	21.4	14.3	23.2	15.0	13.0	13.7	19.6	19.3	14.9
SE*Cb*	23.7	34.8	31.0	17.4	10.1	16.7	15.3	12.0	23.0	28.3	24.3	19.9	11.2	20.6	22..9	18.9
L*Cb*	19.9	28.1	26.6	20.5	9.8	17.0	15.4	11.7	19.7	23.0	20.3	18.0	11.7	18.9	18.3	16.7

Extract code: Fp*Ad*- Fruit Pulp *Adansonia digitata;* L *Ad*- Leaf *Adansonia digitat;* Fp*Cc* –Fruit Pulp *Crescentia cujete;* L*Cc*- Leaf: *Crescentia cujete;* SE *Cb*- Seed Embryo *Ceasalpinia bonduc;* Leaf *Cb* – Leaf *Ceasalpinia bonduc*

Mesocarp extracts isolated from the fruits of trees using different solvents curbed the growth of the coliform species *Escherichia coli* to a notable extent. Between the two trees, *A. digitata* extracts offered better results than *C. cujete* (Plate-3a&b). Impressive results were available also in seed embryo extractions of the legume, *C. bonduc* (Plates- 3c). Microbe free zones caused by *A. digitata* extracts and *C. bonduc* extracts appeared were more conspicuous. In a comparative scale aqueous extracts had lesser impact than solvent extracts. Extracts from fruit pulp were freely soluble in water. Effective extracts created wider zones of inhibition and in this sense methanol extracts produced best results in all three species. From comparisons between treatments it can be inferred that *A. digitata* fruit pulp and leaf extracts were active in curbing the growth of this opportunitistic pathogen next only to the seed and leaf extracts of *C. bonduc.*

PLATE -3 ANTIMICROBIAL ASSAYS WITH SELECTED EXTRACTS



Crude extracts of selected species *Adansonia digata (*a, d, g & j*) , Crescentia cujete* (b, e, h & k*) and Ceasalpina bonduc* (c, f, i & l) compared. Each plate with (i) petroleum ether, (ii) chloroform and (iii) methanol fractions of leaf (L), fruit pulp/ mesocarp (M) and seed (S) samples highlighted from selected treatments.

Among gram negative species *Klebsiella* posed a greater recalcitrance than other bacteria. Unlike the solvent extracts from Baobab, calabash and bonduc nut extracts (Plate-3d, e & f), especially the chloroform eluents of *Crescentia cujete* provided best results. Pseudomonas cultures that elicited a green shade at the zone of inhibition (Plate-3g, h, &i) were relatively controlled well than the extracts prepared from fruit pulp of trees rather than the leguminous seed, the bonduc nut. While the gram positive staphylococcus was found hard to be controlled by solvent extracts from either trees, chloroform extracts eluted from the seed source provided most effective results of all treatments tried in this study (Plate-3j, k, l). Comparison made between leaf vs pulp/seed extracts showed that the antimicrobial influence was overlapping. While leaf borne principles a edge over mesocoarp in Baobab, only narrow differences were seen in calabash and bonduc nut. *E. coli* and *Pseudomonas* were checked well with extractions of bonduc nut and baobab than calabash while this trend is seen reversed in *Klebsiella* and *Staphylococcus.*

Effects of plant extracts on E coli

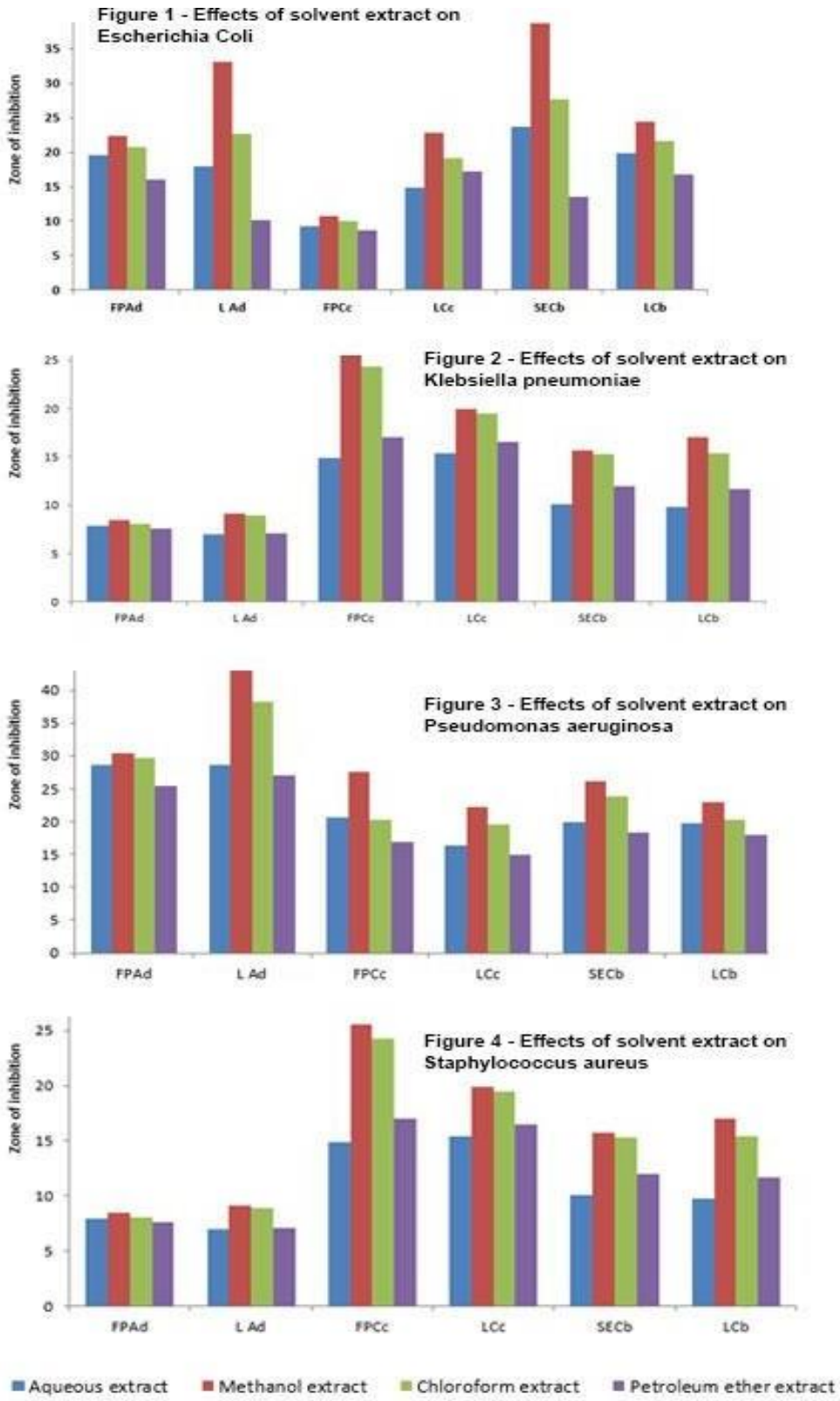
This most common bacillus used as test organism in Kirby and Broyer antimicrobial assays presented mixed responses in different treatments. Of the different sample sources *C. bonduc* methanol extract provided the best results (Fig-1). As the leaf as well as the fruit pulp samples of *C. cujete* offered the least impressive results among all samples, petroleum ether failed to impress as an effective eluting agent in all three plant species. Amidst the discrepancies in each sample class, alcoholic extraction topped the chart to fetch effective antimicrobial substances irrespective of plant and tissue source. Besides providing clear and discrete zone of inhibition in every case, the polar eluent yielded better results than other apolar fractions.

Klebsiella pneumoniae exhibites recalcitrance

When compared with *E. coli*, *Klebsiella pneumoniae* displayed resistance in almost all treatments. It appears that the inoculum of Klebsiella used in this study is relatively more recalcitrant than the other two gram negative forms. Surprisingly extracts of *Crescentia* which evoked a lesser response in *E. c*oli, curbed *K. pneumoniae* the best. With the treatments of *C. bonduc* and *A. digitata* lagging behind, methanol and chloroform extracts from the fruit pulp of *C. cujete* provided good results (Fig-2). Observations made with *Klebsiella* were unique in many ways. In the sassy conducted this microbe exhibited a narrow s zone of inhibition with extract from Baobab. However, the thinner microbe free area created around the wells was not clear and emphatic suggesting a poor control. However results with Calabash extracts were overwhelming in *Klebsiella*. The clear zone in both leaf and fruit pulp samples remained distinct even after prolonged incubation in these treatments. Within *C. cujete* pulp isolates, methanol and chloroform fractions were more effective than aqueous and petroleum ether extractions.

Adansonia digitata is best at work on Pseudomonas aeruginosa

Nevertheless fruit pulp of *A. digitata* used in the study produced outstanding results in curbing the growth of *Pseudomonas* (Fig-3). Antimicrobial assays conducted with the pathogenic strains of *Pseudomonas* species showed that extracts made from both mesocarp leaf samples. *A. digitata* prevented the spread of the bacterial strain over a vast area than any other sample. Substances extracted from the fruits of *A. digitata* offered convincing results in all four forms of extractions. In comparison with *A. digitata*, both *C. cujejte* and *C. bonduc* exhibited only a moderate control. It could be seen that the effects were of varying magnitude in samples eluted from fruit pulp unlike the source leaf there was an even response. In a closer watch of results, *C. bonduc* against *Pseudomonas* could be seen to offer good results with leaf extracts the seed embryo derived fractions.



FPAd –fruit pulp Adansonia digitata; LAd – leaf Adansonia digitata FPCc – fruit pulp Crescentia cujete; LCc - leaf Crescentia cujete; SECb – Seed Embryo Caesalpinia bonduc ; LCb – leaf C. bonduc

Caesalpinia bonduc extracts exert greater control on Staphylococcus aureus

Comparisons made on responses of microbes revealed that *Staphylococcus* was the difficult of all bacterial strains to be controlled in this study. When compared with the gram negative forms, the gram positive cocci exhibited a quicker and better spread indicating pronounced resistance. In the assays, *A. digitata* hardly made an impact. In contrast to the impressive results that this species has elicited in the raking order on gram negative strains, *A. digitata* mesocarp and leaf extracts performed poorly in controlling *Staphylococcus*. *Crecentia* also failed to elicit noteworthy results. In contrast, extracts prepared from *C. bonduc* did reasonably well. Unlike the extracts of two fruit trees, samples prepared using leaves and seed embryos of the legume was helpful in curbing the growth of *Staphylococcus* (Fig- 4). Comparisons made in terms of the zones of inhibition showed that the phytoconstituents eluted from seed sources were more convincing than leaf drawn ingredients.

Charts made for a comparative analysis on the efficacy of extracts with individual strains of microbes provided certain interesting insights (Figures 5-7). Of the two tree species used against *Staphylococcus* strains, *A. digitata* exerted a weak control in almost all forms of extractions. It may be recalled here that the fruit pulp of *Adansonia* offered the most attractive results on two of the three gram negative bacteria tested in this study (Fig-5). *C. cujete* extracts which again failed to impress results on *E.coli* and *Staphylococcus* was yielding gainful indications on *Klebsiella* than other plant species (Fig-6). Both fruit pulp and leaf samples in methanol and chloroform fractions from this species fared better than Baobab and Bonduc in controlling this microbe. Figure-7 presenting the summary responses of antibacterial activity of *Ceasalpinna bonduc* shows that seed extract is effective in controlling the gram positive bacterium more than other plants providing adduce to the preposition that phytochemicals contained in the seed legume can be effectively and diligently used to control virulent pathogenic infections.

DISCUSSION

Cytochemical and anti-microbial investigations pursued at the present instance provides insights on how these plants can be approached to realize their utility. Data gathered from histochemical studies with fresh tissues, and the preliminary phytochemical tests of the eluted fractions of fruit pulp and de-coated seeds show that there is a species and organ specific presence of metabolites. Bacterial assays with four different pathogens lend evidences to suggest that the selected species may find specific uses in treatment of infections that could be caused by pathogens examined here. Attempts made to check for curative chemicals show that fruits and seeds to be store house of macromolecules with additional avenues of fortification with metabolites could confer medicinal utility to the chosen taxa.

The comparative data on phytochemical tests revealed a certain pattern in housing of phytoconstituents. While the primary metabolites present widely in somatic tissues, exit and secondary phytochemicals are sparsely seen in fruit pulp. Cotyledon considered an embryonal leaf showed a deviation from this trend with a liberal presence of phytochemicals in Bonduc nut, unlike the msocarp/fruit pulp in trees. It appears that the perenating seed is vested with many different types of metabolites than the fruit pulp suggesting a predisposed histological and morphogenetic orientation. Scientific screening of raw drugs begins with qualitative tests and pharmacognisitic studies prescribed for scientific validation (Djordjevic, 2017). Although there could be parallels drawn between the two large non-conventional fleshy fruits, results on the chemical profile of the pulp *Adansonia* and *Crescentia* were not truly overlapping.

Figure 5 - Comparative effects of Adansonia digitata on bacterial strains

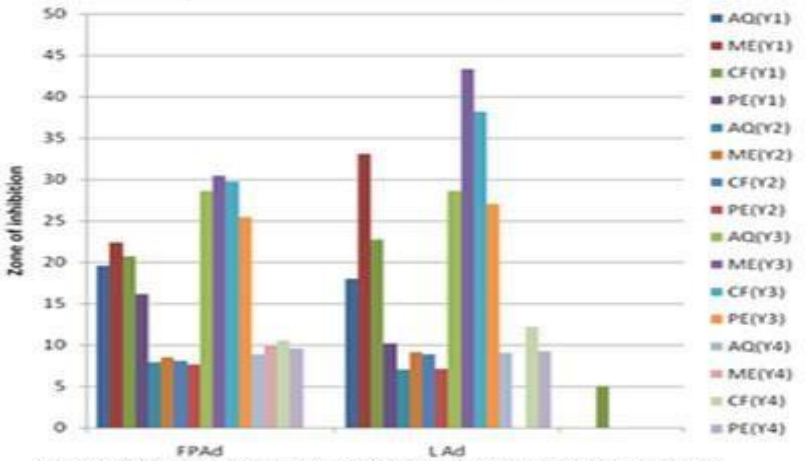


Figure 6 - Comparative effects of Crescentia cujete on bacterial strains

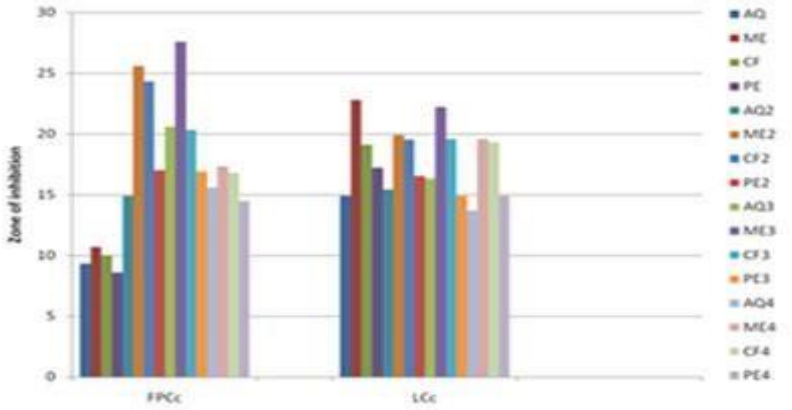
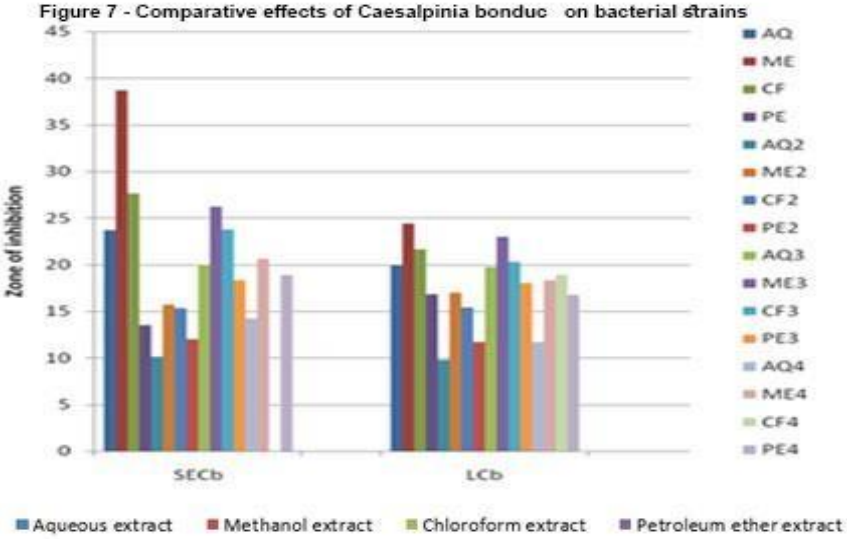


Figure 7 - Comparative effects of Caesalpinia bonduc on bacterial strains



Crude extracts of the seed legume *Ceasalinnia bonduc* more profuse and elaborate in phytochemical milieu reflecting its expansive self-protecting abilities and therapeutic potential (Khan, 2015). Comparisons within the constituent tissues and organs of the given plant form where extracts are drawn helps in arriving at certain generalizations. That the phytochemicals are present differently in different organs and are seen stored in histologically distinctive entities lend credence to the case specific recommendations of drug advocacy in herbal medicine (Van Lommel, 2003). Herbal medical practitioners believe that strict adherence to rules of material collection, medicine preparation and strictures on drug intake and dietary discipline tells upon the efficacy of treatment (Shukla and Sinclair, 2009).

It is also common that in phytotherapy manifold utilities can be harnessed from single plant source. Therefore in raising a prescription crude drugs will have to be subjected to stringent assessments (Mukherjee, 2015). It is here that the preliminary information gathered at the present instance on the cytochemical aspects and the anti-bacterial studies looks relevant. In discussing the safety aspects of crude drugs, it is suggested pharmacognosic and histochemical characterizations are mandatory (Sahoo *et al.,* 2010; Giri *et al.*, 2018). Although not exhaustive, present observations can be construed to add adduce to the previous claims that the three plants can be considered therapeutic.

Registering a positive response for glycosides, flavonoids and tannins, the fruit pulp sample of Baobab (*Adansonia digitata*) presented itself as a potential repository of therapeutic chemicals. As a substance built on sugar moiety glycosides are reported to be a part of storage reserves in this species and many perennials (Li *et al.,* 2017). Since glycosides are considered sugar derivatives, it is not surprising to see them stored in fruits since the latter functions as a principal sink upon defoliation of the tree in summer (Kamatou *et al.,* 2011). Glycosides assume plant protective and nutritive functions in storage and rejuvenating plant organs) and therefore it is not common to expect them in the fruits and seeds (Braca *et al.,* 2018; Sokeng *et al.,* 2019).

It is interesting to note that unlike the regular edible fruits and vegetables used for food, nutrition and general purposes, fruits of *Adansonia* and *Crescentia* studied here with a slim but strong metabolite milieu that can exert specific antimicrobial effects. Our present results are in conformity with data on fruits of the two trees reported with glycosides, flavonoids and tannins in *A digitata* (Eltahir et al., 2019) and phytostreols, fatty acid and fixed oils in *Crescentia* (Mackenzie Theis et al., 2017: Islam, et al., 2019). It is appears that *Adansonia* fruit pulp rich with gums that helps *Adansonia* to evade hot summer (Zinyama et al., 1990). Conversely Tree Calabash (*Crescentia cujete)* which prefers lodging fatty acids in mesocarp as strategy to tide over changing seasons provides the said chemicals attributed with resilience and curative potential (Ejelonu *et al.,* 2011; Saravanaraj *et al.,* 2017).

Studies on the leaves as well as seeds of *Caesalpinia bonduc* shared traits in common with either tree. As a much acclaimed medicinal than the trees, the legumnoius straggler offers extracts of the prominent seeds from pods which showed the clear positive indications for glycoisides, phytosterols, fixed oils, free aminoacids, and notably lacked tannin. In contrast to the pulp of trees that contained less diversified chemicals constituents, solvent elution of the seed legume revealed the presence of several other metabolites namely starch, lipids, amino acids and proteins, alkaloids, phytosterols and fatty acids. This boarder spectrum of phytochemical array could be the reason for the wider uses and accomplishments attributed to this species (Smebiring *et al.*, 2018; Gadakh *et al.*, 2020).

The positive results in bacteriological assays involving 96 different treatments opens up interesting discussion. Zone of inhibition serving as a reference and indication on the effectiveness plant extract (Holder and Boyce, 1994), it is noticed that microbial strains responded differently in a case specific manner with each extract (Fig. 1-4). Comparative data on the effects of the extracts show that all three plants have potential to quell bacterial growth in

E.coli and *Pseudomonas.* The activity chart comparing the effectiveness of plant extracts with reference to the specificity of eluting solvent shows alcohol fractions to be ideal than others (Parvin *et al.*, 2015). At almost all instances except the seed samples of *Caesalpinia bonduc* where chloroform surpassed, methanol treatments were rated the best (Fig. 5- 7).

Since three of the four tested microbial strains are controlled well by using the extracts prepared from mesocarp, it could be deduced that fruits as natural and inherent reservoirs of nutritive and anti-bacterial substance have invoked positive changes. Earlier observations on the composition of the fruit pulp in the two tree genera (Ejelonu, 2004; Kamatou *et al.*, 2013), perhaps comes in handy in deciphering the good results in this study (Table-3). Anti bacterial activity in general had been ascribed to a diverse list of plant constituents. To cite, essential oils, lignans, tannins, saponins, alkaloids, flavonoids and steroids are commonly recognized with anti-bacterial activity (Pandey and Kumar, 2013).

Results of the present study clearly show that gram positive species is harder to be controlled than the gram negative ones. Like *Klebsiella* that evinced considerable resistance as explained in previous studies (Navon-Venezia etal., 2017; Rowe *et al.*, 2021), the gram positive *Staphlococcus* presented recalcitrance (Plate -3). Of the three species, *Caesalpinia bonduc* recorded to contain free amino acids, fixed oils and fats acids in seed extracts exerted effective control. Lachowic*et al.,* (1998) recognized five different types of basil plants based on the ability to control gram positive and gram negative bacteria with direct correlation to the essential oil content. The effectiveness of seed extracts of *C. bonduc* in the control of *Staphylococcus* could be attributed to fats and fixed oils as among the three sources Crescentia pulp and Bonduc nut extracts elicited this trait.

Past studies on ethnopharmacognosy and dietary aspects of the selected plants has only limited references to the antimicrobial activity. As samples of fruit pulp origin *Adansoina digitata* and *Crescentia cujete* offeredan admirable control, a general recommendation to use them as potent antimicrobial agents can be made in their favor. Even though extracts from fruit pulp and seeds have provided a broader spectrum of control, the specific positive inferences on the active control of *Klebsiella* with *Crescentiacujete* and *Staphylococcus* with *Ceasalpinina bonudc* deserves special mention. With marked presence of glycosides and flavonoids in Baobab, phytosterols and fatty acids in calabash tree, and a combination of these said substances with notable absence of tannins in Bonduc nut it is only fair to conclude that selected botanicals are reliable therapeutics. Detailed follow-up and further analysis canprovide more support to the present claim and the idea that such studies shall fetch alternate and better solutions to deal with epidemics and pandemics that confronts health security.

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