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A COMPARATIVE STUDY ON CHARACTERISATION OF VARIOUS EXTRACTS OF *CARICA PAPAYA* LEAVES

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ABSTRACT

Carica papaya is a tropical and a sub-tropical tree, that is tall and herbaceous with the leaves arranged in a spiral manner around the trunk. The leaves of this tree are huge and palmately lobed. The leaf extracts of *Carica papaya* have been used in the treatment of various diseases including urinary tract infection, dengue, malaria and many others. Preliminary phytochemical analysis of the dried leaf extract using solvents like water, methanol, ethanol and acetone contained alkaloids, flavonoids, terpenoids, anthocyanins, phenolic acids and saponins. A comparable study of the absorption spectra of the column chromatographed extracts with the solvents was made between the fresh and the dried leaf extracts. The graph between O.D (optical density) values and different wavelengths were plotted to evaluate the peaks obtained. Thin layer chromatography (TLC) was used for the separation of the column chromatographed extracts and was observed under UV trans-illuminator. The leaf extracts showed the presence of alkaloids, Anthocyanins, flavonoids, Phenolic acids, saponins and Terpenoids in various solvents. The column chromatographed methanol and ethanol extracts of the leaves showed the peaks at 410 nm and 570 nm, respectively. This may be due to the presence of bioactive compounds in the leaf extracts.

KEYWORDS: *Carica papaya*, Column chromatography, TLC, Bioactive compounds.

INTRODUCTION

Carica papaya is the botanical name of papaya which belongs to the family *Caricaceae*. The classification of the plant is:

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliopsida
Class	:	Magnoliopsida
Order	:	Brassicalis
Genus	:	Carica
Species	:	*Carica papaya*

Carica papaya is a tall herbaceous succulent plant with leaves arranged in a spiral fashion around it but only confined to the top of the trunk. The leaves are present in the lower region of the trunk and fruits are borne. Leaves contain a stalk and are huge in size. The leaves are palmated. The trees are dioecious but monoecious are also available in nature. There are no branches evolving from the main trunk. The flowers are small and wax-like appearance is seen on its petals. They appear on the axils of the leaf and develop into a fruit after getting fertilized. The fruit which is produced by *Carica papaya* is a type of berry and is orange in colour when it gets ripened. Basically the fruit is green in colour when it is raw but when ripe, it gets softer and the colour changes to orange. *Carica papaya* was the first transgenic fruit tree whose complete genome was deciphered.[1]

The origin of the *Carica papaya* tree was originally from Mexico and was native to central and northern South America and has become adapted throughout the Caribbean Islands, many countries of Africa and Florida. Even though the origin is from Southern Mexico, India and Brazil are the top producers of papaya (India-5.5 million tons per year). Both India and Brazil accounts up to about 57% of world's total production in 2013. This accounts to the fact that temperature needed for it to grow is optimum and it is highly frost-sensitive, which is the major limitation in production in the tropical climates. It grows best in a well drained, aerated and rich organic soil with ph 5.5 – 6.7.[2]

The parts of plants serve various purposes for human use. The stem or trunk of the plant is used in rope

production. The papaya fruit and the latex that is extracted from the trunk is used as meat tenderizing agent and the constituent present in it is known as papain. It is a protease enzyme which breaks down the meat fibres and tenderizes it. In countries like Thailand raw papaya and flower is used in cooking. The black seeds are edible and are spicy with a sharp taste, which can be used as a substituent for black pepper. Recent papers suggest that it is used to cure diseases like sickle cell disease**,** poison related renal disorder and helminths disease. The brief discussion of leaf is given further and in some parts of Asia, the tender leaves are steamed and eaten like spinach.[3]

The leaf of *Carica papaya* has many phytochemicals present in them which posses many beneficial aspects in treating various kinds of diseases. The plant leaf contains vitamins, thiamine, riboflavin, ascorbic acid. It contains high amount of calcium, magnesium, sodium, potassium, manganese and iron.[4] The aqueous extract from the leaf was very much effective against all the four virus stereotypes of Dengue (DENV-1, DENV-2, DENV-3 & DENV-4) by increasing the platelet count that helps in recovery of the patient quickly.[5] Increase in the platelet count helps in reduced bleeding and thus avoids progression of the illness of Dengue Haemorrhagic fever.[6] The extracts of papaya leaves were effective for treatment of urinary tract infections.[7] The extract of *Carica* leaves shows anti-inflammatory activity in the rats and has potential anti-oxidant property. It was able to destroy the free radicals in an *in vitro* model.[8] The review on the *Carica papaya* was done for its anti-cancer property and was reported that there were many bioactivities showed for the anti-cancer effects.[9] The leaf of *Carica papaya* is found to have many minerals and other elements. The moisture content was found to be 8% and the ash content was found to be 10%. Also there was calcium and magnesium present in more quantity which plays an important role in many physiological processes. In an experiment, it was seen that the alkaloids of *Carica papaya* were effective against malaria causing agent and the carpain (one of the constituent of the leaf) served as an anti-plasmodial agent.[10] The constituents present in the extract of dried leaf of *Carica papaya* contain phenolic compounds, alkaloids, glyceroids, amino acids, minerals and anti-oxidant vitamins. The presence of alkaloids is the reason that it is effective against the malaria disease. In a report, it showed that green leaf, yellow leaf and brown leaf had different constituents and its effects were also different. The yellow leaf can serve as anti-anaemic agent whereas brown leaves were used as body cleansers.[11]

In an experiment, the non-structural part of the virus i.e., 2B and NS2B-NS3 protease complex was crucial for viral replication and hence this was targeted. It is found that the flavonoid, quercetin is most effective in inhibiting the NS2B-NS3 protease activity. Quercetin is a bioflavonoid which has anti-oxidant properties. Quercetin binds to the active site of the NS2B-NS3 serine protease and inhibits the enzyme, hence inhibiting the viral assembly. Quercetin has potential inhibitory activity against NS2B-NS3 serine protease. ADME and toxicity risk assessment strongly suggest that quercetin has marked antiviral activity against DENV2 virus. Flavonoid, quercetin in *Carica papaya* blocks the viral assembly mechanism of DENV2 virus, hence by showing its anti-viral activity.[12]

The viral protease is comprised of two viral proteins NS2B and NS3 that are associated with each other to form a heterocomplex. The N-terminal region of the non-structural 3 protease forms complex with NS2B cofactor which is essential for viral replication. Prohibiting the processing and release of the viral proteins from the polyprotein precursor would inhibit viral genome replication, thus reducing the number of virion progeny produced. NS2B-NS3 protease acts as a therapeutic agent for antiviral compound, as it has an important role in viral life cycle.[13]

The main objective of project was to identify the phytochemicals present in the papaya leaves in aqueous, methanol, ethanol and acetone extracts for both fresh and dry leaves. It was technically determined by the column chromatography and TLC methods.

Thin layer chromatography is an easy and inexpensive technique by which, the number of components present in the extract is known. The identity of the mixture can be known when the R_f of the component is compared with the R_f of the known component. Additional tests include spraying chemicals on the TLC plates and observing for the colour changes or the plates can be viewed under UV light.[14] TLC is advantageous in two aspects; Firstly, it uses only minute amount of the. Secondly, due to the various components present in the extract, this technique simplifies the identification and isolation of the components which are biologically active.[15]

UV-Vis spectroscopy is one of the types of spectroscopy. The basic principle behind this spectroscopy is that the absorption is caused by the transfer of energy from the radiation beam to electrons that can be excited from lower energy level to higher energy level. The UV-Vis spectrophotometer measures the amount of light absorbed by a sample at each wavelength of the UV and visible regions of electromagnetic spectrums. UV-Vis radiations are of higher energy and shorter wavelength than IR radiation and radio frequency radiation, but not as energetic as X-radiation.

In a UV-Vis spectrophotometer, the beam of light is split. One half of the beam called as the sample beam is directed through a transparent cell containing the solution of the sample being analyzed, and one half is directed through an identical cell that does not contain the sample solution. Data from the UV-Vis spectrophotometer is presented as an absorption

spectrum, which is the graph of wavelength (λ) versus sample absorbance (A) at each wavelength in the spectral region of interest.[16]

MATERIALS AND METHODS

Collection of the leaf sample

The leaves of *Carica papaya* were handpicked from St Joseph's College of Arts, Science and Commerce, Langford road, Bengaluru, Karnataka.

Dry sample preparation

The leaves were air dried for 10 days under shade and ground into fine powder using mortar and pestle in the laboratory and packed in a sealing packet for further investigation.

Phytochemical analysis

To analyse the chemical components present in papaya leaf, two sets of extracts were made use of. One set of extract was made using a fresh leaf and the other set was made using dry leaf powder.

Dry leaf powder was extracted using water, methanol, ethanol and acetone to carry out the phytochemical analysis.

Test for Alkaloids

To the few ml of plant extract, add a few drops of Wagner's reagent. Appearance of brownish-red precipitate indicates the presence of alkaloids[17]

Test for Anthocyanins

To 2 ml of the extract, add 2 ml of 2N HCl and ammonia. Appearance of pink-red which turns to blue-violet indicates the presence of Anthocyanins[18]

Test for Flavonoids

To the few ml of the extract, add a few drops of dilute NaOH followed by the addition of dilute HCl. Appearance of yellow coloured solution which gets colourless with the addition of dilute HCl is observed[17]

Test for Phenolic acids

To the few ml of the extract, add a few drops of ferric chloride solution. Appearance of bluish black colour indicates the presence of phenolics[19]

Test for Saponins

To 0.5 ml of the extract, add 5 ml of distilled water and shake vigorously. Persistence of frothing indicates the presence of saponins[18]

Test for Terpenoids

To 1 ml of the extract, add a 5 ml of chloroform. Add 3 ml of conc. H_2SO_4 along the sides of the test tube to form a layer. Appearance of reddish-brown colour in the interface indicates the presence of terpenoids [19]

Extraction from dried leaves

1 gm of dried and powdered leaves (without any veins) were taken and ground in a mortar and pestle using 20 ml of water, 20 ml of ethanol, 25 ml of methanol and 35 ml of acetone to make four different extracts of the *Carica papaya* leaf respectively.

Extraction from fresh leaves

5 gms of leaves were washed and cut into smaller pieces (without any veins) and ground in a mortar and pestle using 10 ml of water and 15 ml of methanol and filtered using muslin cloth and then later the crude was filtered using Whatman filter paper to make an aqueous and a methanol extract of the *Carica papaya* leaf respectively.

Obtaining optical density (O.D) values for fresh and dried leaf extracts

5 ml of the aqueous extract was taken in a test tube and 5 ml of distilled water was added to it and mixed thoroughly. The same was done with methanol, ethanol and acetone extracts. The O.D values were taken at absorbance of 540 nm using photo spectrometer.

Column chromatography of the leaf extract with methanol

- A column was made for chromatography using silica gel filled in a burette up to the mark of 15.0 cms.
- The column was made wet by pouring little methanol from the mouth of the burette till it reached the tip of burette.
- The crude extracts of methanol of both fresh and dried leaves were taken and poured into two separate columns.
- When the coloured extracts started to emerge out from the burette passing through the silica gel, 10 test tubes were taken (marked as 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) and in each test tube 1ml of extract was collected and stored at room temperature of each of the dried and fresh leaf extracts (methanol extract).
- The same procedure was carried out for the aqueous, ethanol and acetone extracts.

Thin Layer Chromatography of the extracts:

- For TLC of the extracts, 50 gms of silica gel was mixed in 100 ml of water and mixed evenly. 10 clean and dry glass plates were taken (marked as 1, 2, 3, 4, 5, 6, 7, 8, 9, & 10) and the evenly mixed silica solution was poured on all the 10 plates and spread evenly using a clean glass rod.
- All the plates were kept at room temperature for 24 hrs so as to evaporate the water from the plates.
- The silica on the plates was activated by incubating them at 60ºC for 20 mins in an incubator.
- The chromatographed extracts of methanol (starting from 1 to 10) were placed as dots on the plates at one end (little above the edge of the plate).
- BAW solution of ratio 12:7:1 was made by mixing 12ml of butanol, 7ml of acetic acid and 1ml of distilled water.

- The 10 plates were placed in the BAW solution for 24hrs.
- The run plates were removed from the BAW solution and were dried at room temperature till all the solvents wee evaporated.
- All the plates were observed under U.V using U.V trans-illuminator for analyzing.

RESULT AND DISCUSSION

1. Phytochemical Analysis.

Table 1: Qualitative analysis for Phytochemical compounds.

Phytochemicals	Water	Methanol	Ethanol	Acetone
Alkaloids	-	+	+	-
Anthocyanins	+	+	+	+
Flavonoids	+	+	+	+
Phenolic acid	-	+	+	+
Saponins	+	+	+	-
Terpenoids	-	-	+	-

From the Table – 1, it was found that the ethanol extract contains high amount of phytochemicals such as Alkaloids, Anthocyanins, Flavonoids, Phenolic acids, Saponins and Terpenoids followed by Methanol, Water and Acetone extracts. It is important to know the importance of each of the phytochemicals as they are found to cure various diseases. Many phytochemicals have been found in the plants and the food we eat. Only a few out of them have been listed above. Their uses are as follows:

a) **Alkaloids:** Many alkaloids although poisonous, have physiological effects which render them valuable as medicines. They have various uses in the medical field. They are used as relaxants, pain reliefs, pupil dilators, to cure muscle diseases, etc. A few alkaloids such as cocaine are anaesthetic.

b) **Anthocyanins:** Anthocyanins are used as food additives. These are vaculor, water-soluble pigments which appear red, purple or blue depending on the pH. Anthocyanins act as sunscreens, protecting the cells from high-light damage by absorbing blue-green light and ultraviolet light, hence protecting the tissues from photoinhibition or high-light stress. Anthocyanins also act as strong antioxidants.

c) **Flavonoids:** flavonoids are a class of plant and fungus secondary metabolites. Flavonoids inhibit the pro-inflammatory activity of enzymes involved in free radical production.

d) **Phenolic acids:** Phenolic acids are a group of phytochemicals called polyphenols. They may be beneficial to your health because they work as antioxidants that prevent cellular damage due to free-radical oxidation reactions. It also decreases risk of cancer and other chronic diseases.

e) **Saponins:** Saponins are phytochemicals which can be found in most vegetables, beans and herbs. The best known sources of saponins are peas, soybeans, and some herbs with names indicating foaming properties such as soapwort, saoproot, soapbark and soapberry. Saponins have many health benefits. They cause a reduction of blood cholesterol by preventing its re-absorption. Studies have shown that saponins have antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. Plants produce saponins to fight infections caused by parasites. They also reduce risk of cancer and heart diseases.

f) **Terpenoids:** Terpenoids are also known as isoprenoids. They are used for the treatment of cough, arthritis and syphilis.

2. Comparison of graph between aqueous and methanol extract of fresh leaf.

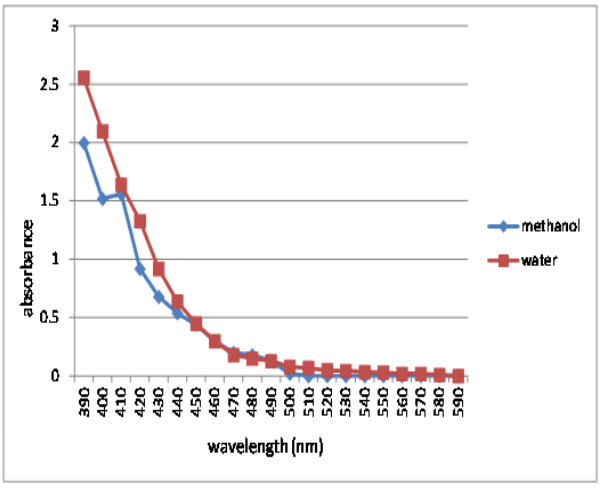

Fig – 1: Comparison of graph between aqueous and methanol extract of fresh leaf.

From the Fig.1, in the fresh leaves extract of methanol, the O.D values vs. different wavelength graph gives a peak at 410nm, where the O.D value is about 1.55. There is a break in the graph between 480nm to 500nm which is due to the high value that was out of range to be read by the photo spectrometer. Also there is a break between 520nm to 550nm. Then the graph kept on reducing till zero.

On the other hand, graph of fresh leaf extract of water between the O.D values vs. different wavelength shows no peak as such but there is a break between 410nm to

430nm which is again due to the high value. Also there is a break between 480nm to 500nm and 520nm to 550nm.

3. Comparison of graph between ethanol, acetone, methanol and aqueous extract of dried leaf:-

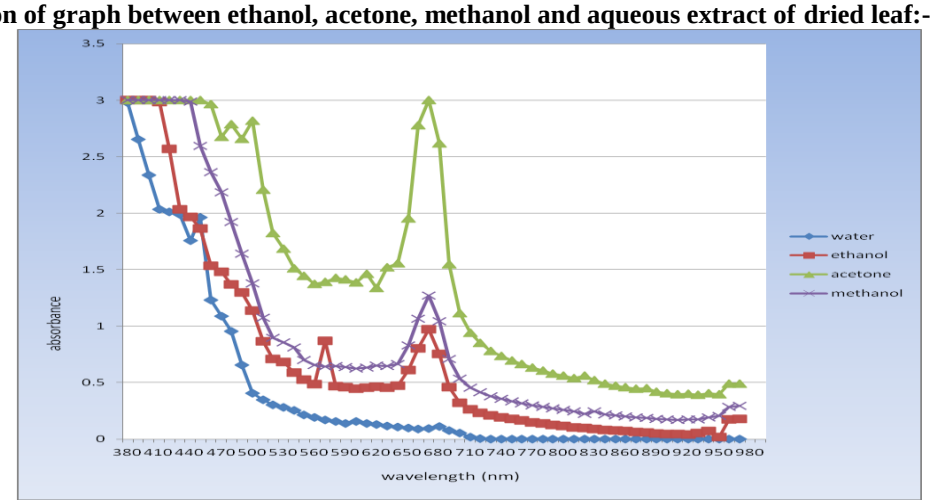

Fig 2: Comparison of graph between ethanol, acetone, methanol and aqueous extract of dried leaf.

From the Fig.2, in the aqueous extract of dried leaf, there is a break between 420 nm to 440 nm, 440 nm to 500 nm and 520 nm to 540 nm after which the graph kept on declining down and reached zero.

In case of ethanol, the value from the wavelength 380 nm to 500 nm was too high (>3) but there was a break at 520 nm to 540 nm like in case of aqueous extract. But the interesting thing about these graphs is that there is a peak at 570 nm. Other than that there is a breakage of graph line between 820 nm to 840 nm, 880 nm to 890 nm, 920 nm to 930 nm and 940 nm to 950 nm. At the end of each of the graph there is a slight elevation of the graph line.

In case of acetone and methanol extracts, there is a similar graph obtained. The initial values from 380 nm to 500 nm is high (>3). From 520 nm to 540 nm, 810 nm to840 nm, 880 nm to 890 nm, 910 nm to 920 nm and 950 nm to 960nm there are breaks in the graph line. The graph of acetone extract is quite higher than the graph obtained from the methanol extract. There is a sharp peak at 670 nm in case of both acetone and methanol.

In methanol, ethanol and acetone extract the graph looks similar (except in ethanol there is a peak at 570 nm). Starting with very high values (>3), then reducing gradually, showing break between 650 nm to 700 nm and decreasing almost to zero and again increasing little above zero.

4. Column chromatography of methanol extract of fresh leaf and dried leaf

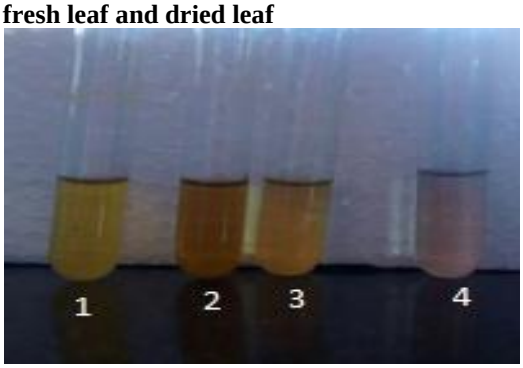

Fig – 3: Column chromatography extracts showing different colors from test tube 1 to 4 (left to right).

In column chromatography extract, the colour of the extract of the first tube has light green color but the second test tube has little dark brown colour. The third test tube shows pale orange color whereas the fourth test tube shows a tinge of violet colour. From 5th to 10th there is no colour shown in case of fresh leaf extract of methanol. Similar colors were observed in case of dried leaf extract of methanol.

5. Thin layer chromatography (T.L.C) of methanol extract of fresh leaves

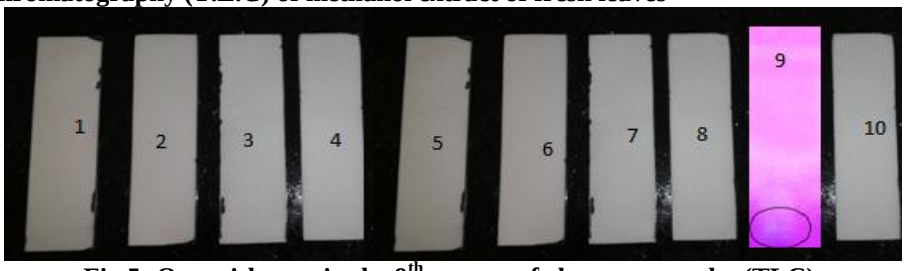

Fig 5: Orangish spot in the 9th extract of chromatography (TLC).

From the Fig.5, the results of the T.L.C of all the ten test tubes extracts in case of fresh leaf extract on analyzing using U.V trans-illuminator shows that in the ninth T.L.C plate, there was a spot of orangish colour observed.

In the fresh leaf extract of methanol, there is a peak at 410nm which indicates that there is a compound which is responsible to give that peak. In the fresh leaf extract of ethanol, in case of column chromatography, there is a peak at 570nm which may a bioactive compound. In all the dried leaf extracts (acetone, ethanol, water and methanol) of column chromatograph there is a peak at 660nm to 680nm. This is because of the compound chlorophyll which shows absorbance at 660nm to 680nm.

CONCLUSION

The phytochemical analysis of the dry powder extract indicated the presence of alkaloids, Anthocyanins, flavonoids, Phenolic acids, saponins and Terpenoids in various solvents. The peaks in fresh leaves extract of column chromatographed methanol and ethanol extract gives an indication that some bioactive compound is present which may belong to the class flavonoids or alkaloids due to which these peak is obtained. This work can be further investigated to identify the actual bioactive compound present which may give a better idea for analyzing and testing it to see if it increases the platelet count.

REFERENCES

1. Bruce, S. and C.A. Peter, 2008. Handbook of Environmental Physiology of Fruit Crops. 1 Ed. 217.
2. Nephroprotective activities of the aqueous seed extract of Carica papaya Linn. in carbon tetrachloride induced renal injured Wistar rats:a dose-and time-dependent study Biology and Medicine. Olagunjua, JA., Adeneyeb, AA., Fagbohunkac, BS., Bisugac, NA., Ketikuc, AO., Benebod, AS.,Olufowobic, OM., Adeoyec, AG., Alimic, MA and Adelekec, AG. aDepartment of Medical Biochemistry, bDepartment of Pharmacology, dDepartment of Forensic Medicine and Pathology, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria. cDepartment of Biochemistry, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Ikenne, Ogun State, Nigeria, 2009; 1(1): 11-19.
3. Effectiveness of dried Carica papaya seeds against human intestinal parasitosis: a pilot study. Okeniyi, J.A., Ogunlesi, T.A., Oyelami, O.A. and Adeyemi, L.A., 1Department of Paediatrics and Child Health, College of Health Sciences, Obafemi Awolowo University, Ile-Ife; and Departments of 2Paediatrics and 3Microbiology and Parasitology, Wesley Guild Hospital, Ilesa, NigeriaJournal of medicinal food, 2007; 10(1): 194-196.
4. Phytochemical and nutrient evaluation of Carica papaya (pawpaw) leaves. Ayoola, P.B. and Adeyeye, A., Department of Science Laboratory Technology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria Ijrras, 2010; 5(3): 325-328.
5. Research Note Dengue serotype surveillance among patients admitted for dengue in two major hospitals in Selangor, Malaysia. Ab-Fatah, M., Subenthiran, S., Abdul-Rahman, P.S.A., Saat, Z. and Thayan, R., 1Virology Unit, Institute of Medical Research, Kuala Lumpur 2Bioassay Unit, Institute of Medical Research, Kuala Lumpur 3Department of Molecular Medicine, Faculty of Medicine; 4UM Centre for Proteomics Research; University of Malaya, Kuala LumpurTropical biomedicine, 2015; 32(1): 187-191.
6. Utility of multiplex reverse transcriptase-polymerase chain reaction for diagnosis and serotypic characterization of dengue and chikungunya viruses in clinical samples. Mishra, B., Sharma, M., Pujhari, S.K., Ratho, R.K., Gopal, D.S., Kumar, C.N., Sarangi, G., Chayani, N. and Varma, S.C., Diagnostic microbiology and infectious disease, 2011; 71(2): 118-125.
7. In-vitro sensitivity pattern of some urinary tract isolates to Carica papaya extracts. Yusha'u, M., Onuorah, F. C. and Murtala Y, Department of Biological Sciences, Bayero University, P. M. B. 3011, Kano 2Department of Science Laboratory Technology, Hussaini Adamu Federal Polytechnic, Kazaure Bayero Journal of Pure and Applied Sciences, 2009; 2(2): 75-78.
8. Reduction of hydrogen peroxide–induced erythrocyte damage by Carica papaya leaf extract. Okoko, T. and Ere, D., 1Biochemistry Programme, Department of Chemical Sciences, Niger Delta University, PMB 71, Wilberforce Island, Bayelsa State, Nigeria 2Pure and Applied Chemistry Programme, Department of Chemical Sciences, Niger Delta University, PMB 71, Wilberforce Island, Bayelsa State, Nigeria; Asian Pacific journal of tropical biomedicine, 2012; 2(6): 449-453.
9. Aqueous extract of Carica papaya leaves exhibits anti-tumor activity and immunomodulatory effects. Otsuki, N., Dang, N.H., Kumagai, E., Kondo, A., Iwata, S. and Morimoto, C., a Division of Clinical Immunology, Advanced Clinicl Research Center, The Institute of Medical Science, The University of Tokyo, Japan, b Division of Hematology/Oncology, The University of Florida, Gainesville, FL, USA, c Kondo Hospital, 1-6-25, Nishishinhama, Tokushima, Tokushima 770-8008, Japan, d Department of Rheumatology and Allergy, Research Hospital, The Institute of Medical Science, The University of Tokyo, Japan. Journal of Ethnopharmacology, 2010; 127(3): 760-767.
10. Quantification of the antiplasmodial alkaloid carpaine in papaya (Carica papaya) leaves. Julianti, T., Oufir, M. and Hamburger, M., 1 Division of Pharmaceutical Biology, University of Basel, Basel, Switzerland, 2 Faculty of Pharmacy, Pancasila

University, Jakarta, Indonesia Planta medica, 2014; 80(13): 1138-1142.
11. Atta, K. Bonsu. "The Power of Garlic", Cardiovascular disease prevention Association, Buea, Cameroon, 1999; 72.
12. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants; Jigna Parekh and Sumitra Chanda; Phyochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot – 360 005, India; African Journal of Biomedical Research, Accepted, 2007; 10: 175-181.
13. Dengue and Its Phytotherapy: A Review. Kumar Sarangi, M. and Padhi, S., Assistant Professor, Department of Pharmacy Sardar Bhagwansingh PG Institute of Biomedical Sciences and Research, Balawala, Dehradun, Uttarakhand, India, 248161. International Journal of Pharmaceutical and Phytopharmacological Research, 2017; 4(1): 37-46.
14. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts; S. Sasidharan, Y. Chen, D. Saravanan, K.M. Sundram, L. Yoga Latha; Institute for Research in Molecular Medicine (INFORM), Universiti Sains Malaysia, Minden 11800, Malaysia., Centre for Drug Research, University Science of Malaysia, 11800 Minden, Pulau Pinang. Malaysia., School of Pharmacy, AIMST University, Jalan Bedong-Semeling, Batu 3 ½, Bukit Air Nasi, Bedong 08100, Kedah, Malaysia; Afr J Tradit Complement Altern Med., 2011; 8(1): 1-10.
15. A Bioautographic Agar Overlay Method for the Detection of Antifungal Compounds from Higher Plants; L. Rahalison1, M. Hamburger1, K. Hostettmann1, M. Monod2 and E. Frenk2; 1Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, CH-1015 Lausanne-Dorigny, Switzerland, 2Laboratoire de Mycologie, Service de Dermatologie, Centre Hospitalier Universitaire Vaudois, CHUV, CH-1011 Lausanne, Switzerland; Phytochemical Analysis, 1991; 2: 199-203.
16. Biochemical constituents in leaf of Carica papaya-Ethnomedicinal plant of Kachchh region. Vyas, S.J., Khatri, T.T., Ram, V.R., Dave, P.N. and Joshi, H.S., 1Department of Chemistry, KSKV Kachchh University, Bhuj – 370 001, Gujarat, India 2Department of Chemistry, Saurashtra University, Rajkot – 360 005, Rajkott, IndiaInternational Letters of Natural Sciences, 2014; 7.
17. Evaluation of Phytochemical Constituents, Antibacterial Activities and Effect of Exudate of Pycanthus Angolensis Weld Warb (Myristicaceae) on Corneal Ulcers in Rabbits; DN Onwukaeme1, TB Ikuegbvweha1 and CC Asonye2; 1Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City,2 Department of Optometry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria; Tropical Journal of Pharmaceutical Research, June, 2007; 6(2): 725-730.
18. Screening of Medicinal Plants for Secondary Metabolites; N. Savithramma, M. Linga Rao and D. Suhrulatha; Department of Botany, Sri Venkateswara University,Tirupati-517 502, Andhra Pradesh, India; Middle-East Journal of Scientific Research, 2011; 8(3): 579-584.
19. Qualitative and Quantitative Phytochemical analysis of Moringa concanensis Nimmo; K. Santhi and R. Sengottuvel; Department of Botany, Arignar Anna Government Arts College, Namakkal-637 002, Tamil Nadu, India; International Journal of Current Microbiology and Applied Sciences, 2016; 5(1): 633-640.