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PHARMACOGNOSTIC, PHYTOCHEMICAL, IN-VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY STUDIES ON LEAVES OF *Carica papaya.*

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ABSTRACT
The leaf of *Carica papaya* is also almost available in every season. Exploration of medicinal values of plant like papaya becomes very effective and becomes practically application. The studies included the macroscopic, microscopic, phytochemical screening, antioxidant, and antibacterial activity evaluation of leaves of *Carica papaya*. The microscopic study of leaf showed the presence of epidermis (upper and lower) lower epidermis consist numerous stomata but not in upper epidermis. Beneath epidermis a layer of palisade cells and spongy cells are present. Spongy cells consists rosette crystal. Pith is hollow. The epidermal layer of leaf shows the presence of anomocytic type of stomata. The result of the study revealed that the *Carica papaya* leaves methanolic extract consist large numbers of phytochemicals i.e. alkaloid, glycoside, tannin, terpinoid, saponin, cardiac glycoside, anthraquinone glycoside, flavonoid, phenol and protein. The study shows that the methanolic extract of *Carica papaya* has significant antioxidant activity with highest TPC (41.453 mg GAE/G) and TFC 32.42 mgQE/g). TPC was measures by using Folin-ciocalteau's reagent and TFC of *Carica papaya was* measured by colorimetric assay. The TPC and TFC of the extracts were expressed as milligram of Gallic Acid Equivalent per gram of extracts i.e. mg GAE/g extract and milligram of quercetin equivalent per gram of extract i.e. mg QE/g extract respectively. Antibacterial activities of hexane and methanol extract of *Carica papaya* against the tested stamps of microorganisms were found to be non-indicative. Only the antibacterial activity of ethyl acetate extract *Carica papaya* was found to indicative against *Salmonella typhi.*

KEYWORDS: *Carica papaya*, antioxidant activity, total phenolic content, total flavonoid content, antibacterial activity.

INTRODUCTION
In recent years, traditional medicine has made a comeback for a variety of reasons including side-effects and toxicity of modern synthetic drugs, evolution of multi-drug resistance microorganisms, and the inability of modern medicine to find effective cures for a number of diseases. More than 70% of the developing world's population now depends on traditional medicinal system, otherwise known as complementary or alternative systems of medicine.[1] It is a fact that plants used by indigenous peoples in their traditional medicinal systems are forming the sources of many important new pharmaceuticals.[2]

For a very long time, plants have played an important role in the treatment of many diseases especially in the developing countries. World health organization (WHO) recommends application of traditional medicine in medical health services system. Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components.[3]

Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases.[4,5] Originally derived from the southern part of Mexico, *Carica papaya* is a perennial plant, and it is presently distributed over the whole tropical area. In particular, *Carica papaya* fruit circulates widely, and it is accepted as food or as a quasi-drug. Many scientific investigations have been conducted to evaluate the biological activities of various parts of *Carica papaya*, including fruits, shoots, leaves, rinds,

seeds, roots or latex. The leaves of papaya have been shown to contain many activecomponents that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenicglucosides and glucosinolates.[6]

MATERIALS AND METHODS

Plant material

The plant material i.e. leaves of *Carica papaya* were collected from Bharatpur-7, Chitwan, Nepal and identified as *Carica papaya* at National Herbarium and Plant Laboratory, Godavari, Lalitpur.

Chemical and apparatus

Chemicals used were hexane, ethyl acetate, methanol, 1, 1-diphenyl-2-picryl hydrazyl(DPPH) (Hi-Media), ascorbic acid, quercetin dehydrate, gallic acid, ofloxacin and apparatus used were Microscope, UV-Visible Spectrophotometer (SHIMAZU) and Rotary shaker (Associated Scientific Technologies, Delhi, India).

Pharmacognostic studies

The pharmacognostical study of the *Carica papaya* was performed to find out the characteristic morphological and anatomical features of crude drug material, which helps in identification, prevents the adulteration. Pharmacognostic study includes macroscopic and microscopic study where macroscopic study was performed by observation of the external features such as color, odor, taste, surface texture, fracture etc. as sensed by organoleptic evaluation[7]. Microscopic study was performed by cutting the transverse section of leaf and stem and was observed under the microscope to identify different anatomical feature with special focus to presence of distinct cells, tissues and crystals and their arrangement. For microscopic study permanent slide were prepared.

Processing of samples Plant materials collected were dry-cleaned and the foreign organic material (FOM) was separated. They were shade dried and grinded to obtain the powder with the help of electric grinder.

Preparation of extract

The powder form of sample of 14.0g was taken and subjected to soxhlet extraction. Three different solvent were chosen to run extraction process as per their polarity i.e Hexane, ethyl acetate and methanol. Different liquid extract obtained were dried separately using Rotavapour drier below 40oc and solid extracts were preserved in refrigerator at 4oc. Extractive value of each extract were determined.

Phytochemical Screening

Phytochemical screening included the qualitative tests performed by color reactions and quantitative estimation includes TPC and TFC determination of different extract of plant.

Qualitative phytochemical screening

The phytochemical screening was done to identify the main group of chemical constituents present in different extracts by their color reactions with different reagents. Each extract was subjected for glycoside, alkaloids, tannins, saponin, terpinoids, carbohydrate, cardiac glycoside, anthraquinone glycoside, protein, flavonoid, phenol.[8, 9]

Quantitative phytochemical screening

- **Total polyphenolic content determination**

The total polyphenolic content of the extract were measured using Folin-ciocalteau's reagent. Briefly 0.5ml of extract (5mg/ml) was separately mixed with Folin-ciocalteau's reagent (5ml, 1:10 v/v diluted with distilled water and aqueous sodium carbonate (Na2co3, 4ml, 1M) solution. Then the mixture was allowed to stand for 15 minutes at room temperature. The absorbance of the reaction mixture was measured at 765 nm using spectrophotometer; Gallic acid was used for constructing the standard curve (10 to 80µg/ml) and total polyphenolic compound concentration in the extracts was expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g) of extract.[10, 11]

- **Total flavonoid content determination**

Total flavonoid content of the extracts was determined according to the colorimetric method. Briefly 0.5 ml of each extract (50mg/ml) was separately mixed with 1.5 ml of methanol, 0.1 ml of almunium chloride (AlCl3, 10%). Subsequently 0.1 ml of 1M potassium acetate and 2.8 ml distilled water was added to each test tube and reaction mixture was allowed to stand for 30 minutes. The absorbance was measured at 415 nm with UV-visible spectrophotometer. Quercetin was used for construction of standard curve (10 - 50 mcg/ml) and the total flavonoid compounds concentration in the extracts was expressed as milligram of Quercetin equivalent per gram of dry weight (mg QE/g) of extract.[10,11]

Antioxidant Activity by DPPH Scavenging

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant. The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) according to the previously reported method. Briefly, 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (5µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml). The mixtures were shaken vigorously and allowed to stand in dark room at temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (UV-1800 SHIMAZU). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging

activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) =

(A0 -A1)/A0)*100

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

The % scavenging was then plotted against concentration and regression equation was obtained to calculate IC50 (Micromolar concentration required to inhibit DPPH radical formation by 50%) values.[12]

Antibacterial screening of extracts

Antibacterial activity of hexane, ethyl acetate and methanol extracts of *Carica papaya* were determined by the cup diffusion method on nutrient agar medium. Cups are made in nutrient agar plate using cork-borer of 6mm. Inoculums containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50µl of the each extract were placed in the cups made in inoculated plates, the treatments also included 50µl of DMSO which served as negative control. All the plates were incubated for 24 h at 37°C and zone of inhibition if any around the wells were measured in mm (millimeter). Antibiotics (50µg) ofloxacin was used as reference to determine the sensitivity of each bacterial species tested.

Microorganism taken were; Gram positive organism: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (clinical isolated) and Gram negative organism: *Escherichia coli* (ATCC 259222), *Salmonella typhi* (clinical isolated).[13,14]

Determination of minimum bactericidal concentration (MBC)

Extract Zone of having inhibition (ZOI) >12 mm was subjected to MBC test. Freshly prepared nutrient broth was used as diluents. Crude extract was diluted by two fold serial dilution method. 13 tubes were taken. 50µg/ml of the standard culture inoculums was added to each test tube except the negative control tube. All tubes were incubated at 37°C for 24 h. The tube content was subculture in fresh nutrient agar separately and MBC was determined as that showing no growth.[14]

Data analysis

Data were analyzed using MS-EXCEL 2010. Quantitative tests were conducted in triplicate. The data was presented in form of mean ± SD (standard deviation).

RESULTS AND DISCUSSION

Foreign organic material determination

Weight of FOM was found to be 0.26g in total 250g plant materials. Calculated FOM value was 0.104%.

Macroscopic study

Color: dark green, odour: characteristic, taste: bitter, surface character: leaves of papaya are usually larger and arranged spirally having long stem and their blades are split into 7-11 main lobes are usually veins are deeply palmated and serration are shallow.

Microscopic study

Transverse section of *Carica papaya* leaf lamina shows the barrel shaped, single layerd, wavy cuticularized upper epidermis which is surrounded by collenchyma and sclerenchyma. Lower epidermis consists of numerous stomata but not in upper epidermis. Type of stomata present in it is anomocytic type. Beneath the upper epidermis single layer of palisade cells and 4 to 5 layers of spongy cells are present. In spongy cells the rosette calcium oxalate crystals are present. Mesophyll is differentiated into upper palisade and lower spongy cells. In mid rib region a broad band of collenchyma is present between the epidermis and vascular bundle. The central portion represent by hollow region surrounding numerous distinct vascular bundles which is articulated with lactiferous canals. Vessels of vascular bundle are distinct. Diameter of cells present in T.S of leaf is: epidermis 65.8µm, palisade 173.9µm, parenchyma 61.1µm, rosette calcium oxalate crystal 72.38µm, vessel 83.033µm and lactiferous canal 341.126µm measured by microscope fitted with ocular micrometer at 10 xs.

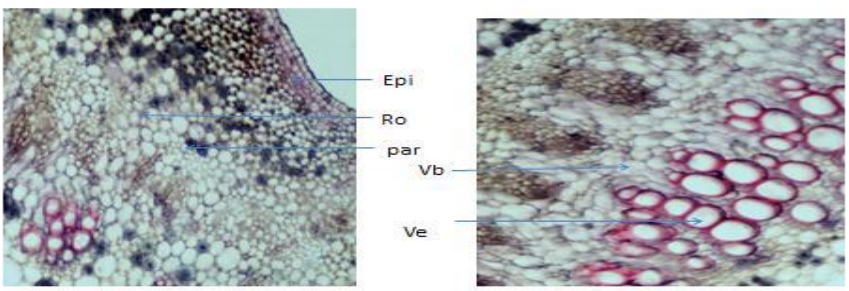
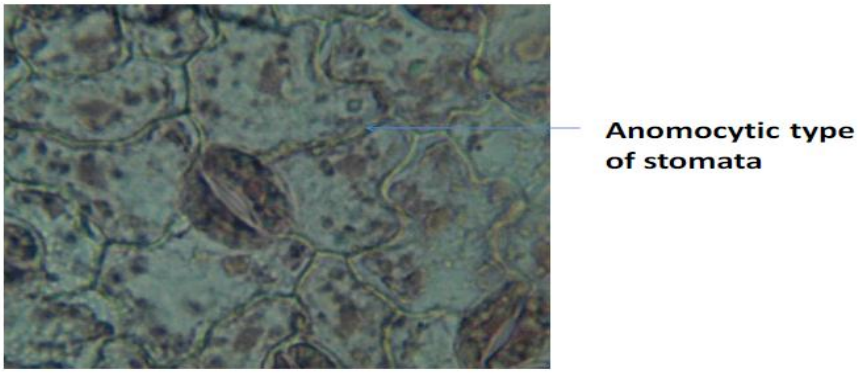


Figure 1: Transverse section of leaf of Carica papaya showing the presence of **Epi**- epidermis, **Ro**-rosette calcium oxalate crystal, **Par**- parenchyma, **Vb**- vascular bundle, **Ve**- vessel.



lower epidermis of leaf *Carica papaya* showing the presence of stomata.

Qualitative phytochemical testing

Phytochemical Screening of different extracts of *Carica papaya* showed the presence of different group of active constituents in hexane, ethyl acetate and methanol extracts. The results obtained were tabulated as follows. As per result hexane extract contained alkaloids, tannin, carbohydrates flavonoid, phenol, protein and glycosides. The ethyl acetate extract contained tannins, flavonoid, phenol, protein and carbohydrates. And methanol extract contained saponnins, tannins, terpinoids, carbohydrate, flavonoid, phenol, glycosides, protein cardiac glycosides and anthraquinone glycosides.

Table 1: Qualitative phytochemical testing results

S.N	Test	Hexane	Ethyl acetate	Methanol
1	Alkaloids	+	+	+
2	Saponinns	-	-	+
3	Tannins	+	+	+
4	Terpinoids	-	-	+
5	Carbohydrates	+	+	+
6	Flavonoid	+	+	+
7	Phenol	+	+	+
8	Glycosides	-	-	+
9	Cardiac glycosides	-	-	+
10	Anthraquinone glycosides	-	-	+
11	Protein	+	+	+

Note: presence '+'; absence '–'

- **Total polyphenolic content determination**

Calibration curve of standard Gallic acid was obtained by Microsoft Excel 2010 where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure:

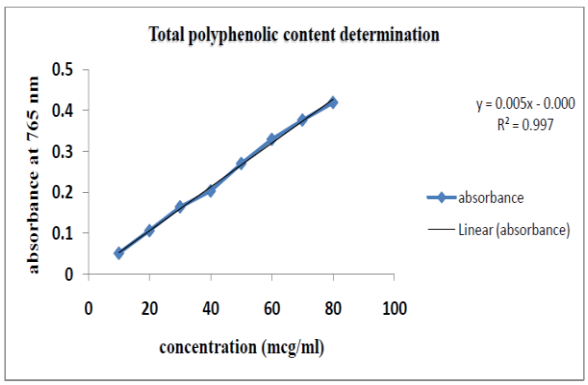


Figure 2: calibration curve of Gallic Acid

Calculation of total polyphenolic content of the extract was done by using calibration curve equation: y=0.0053x-0.0007, R2= 0.9976 obtained by plotting calibration curve of standard Gallic acid where y was the absorbance and x was the concentration. The following bar diagram shows the total phenolic content of different extracts as mg Gallic acid equivalent (GAE) per gram.

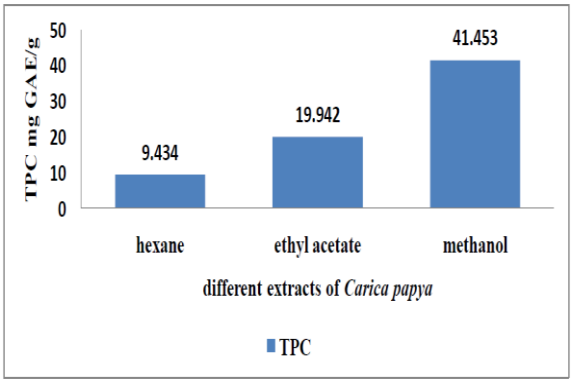


Figure 3: TPC of different extract of *Carica papaya*

- **Total flavonoid content determination**

Calibration curve of standard Quercetin was obtained by Microsoft Excel 2010 where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure:

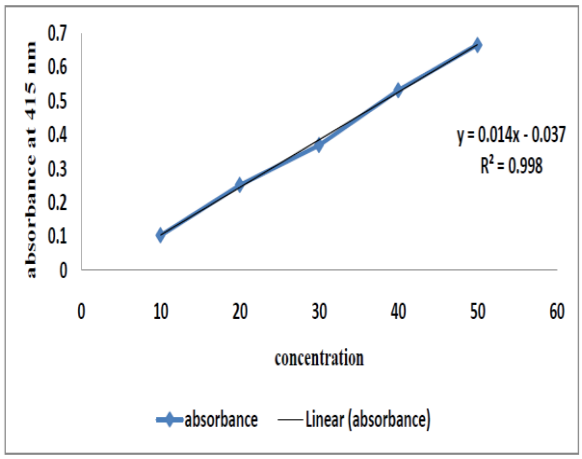


Figure 4: Calibration curve of Quercetin

Calculation of total flavonoid content of the extract was done by using calibration curve equation: y=0.0141x-0.03727, R2= 0.9984 obtained by plotting calibration curve of standard Quercetin where y was the absorbance and x was the concentration. The following bar diagram shows the total flavonoid content of different extracts as mg Quercetin equivalent (QE) per gram;

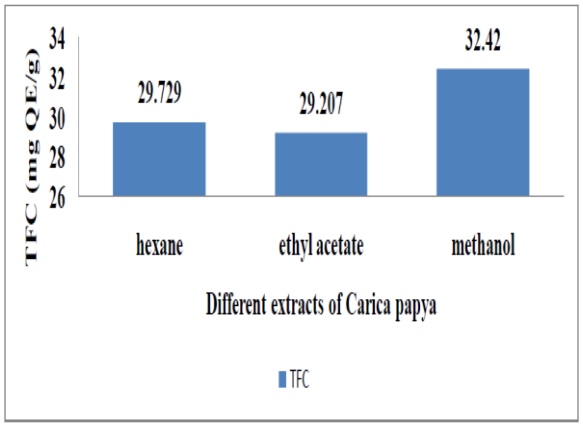


Figure 5: TFC of different extract of *Carica papaya*

Antioxidant activity test

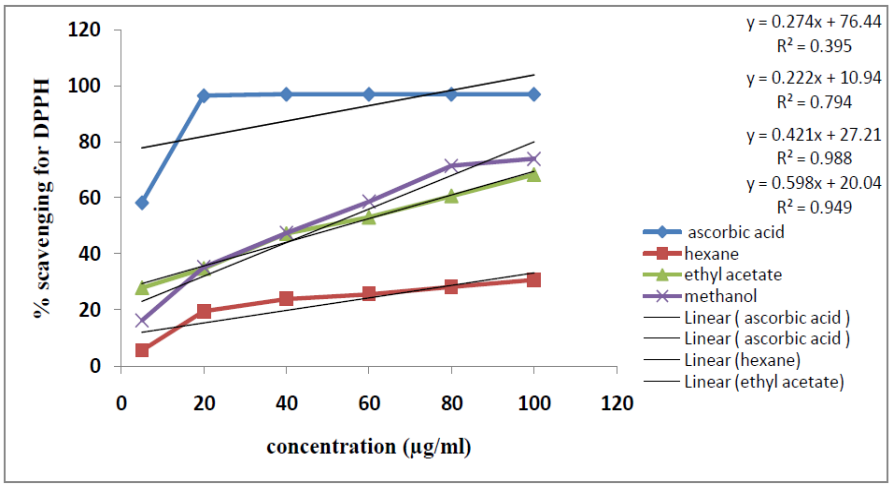


Figure 6: antioxidant activity of different extract of *Carica papaya*

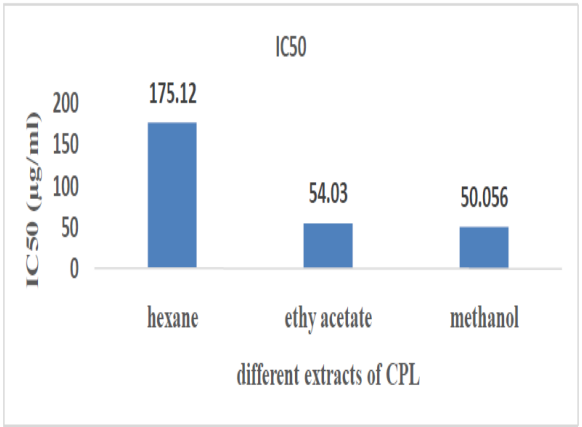


Figure 7: IC50 values of different extract of *Carica papaya*

IC50 values of tested samples were in order: methanolic extract < ethyl acetate extract < hexane extract of *Carica papaya* leaves.

Antibacterial testing

The following table shows the result of preliminary antimicrobial activity of different extracts of *Carica papaya*. Hexane and methanolic extract shows no indicative antibacterial activity against the tested stamps of microorganisms. Only the ethyl acetate extract shows the indicative antimicrobial activity against and *Salmonella typhi* among the tested stamps of microorganisms.

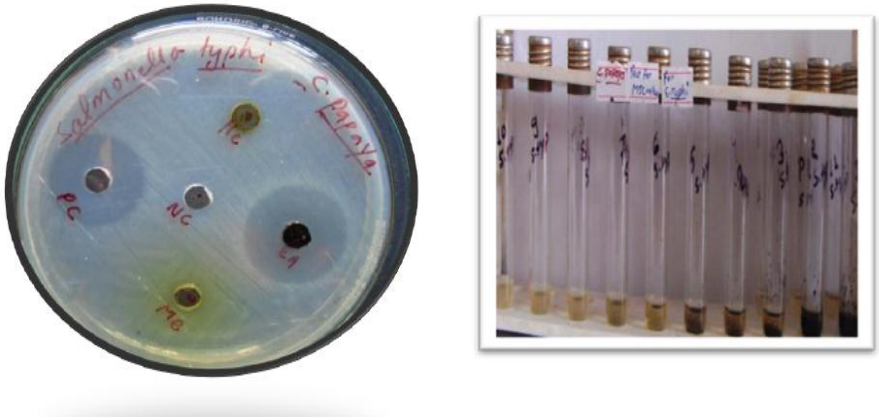


Figure 8: representation of antibacterial activity test & MBC of *Carica papaya*

Table 2: Antibacterial testing results

Microorganism	Zone of inhibition (mm)			
	Hexane extract	Ethyl acetate extract	Methanol extract	Control*
Staphylococcus aureus	-	9	-	19
Bacillus cereus	-	9	-	16
Escherichia coli	-	-	6	29
Salmonella typhi	-	19	-	18

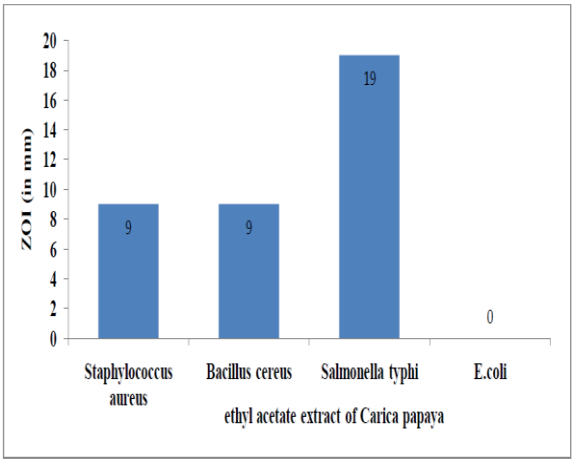


Figure 9: antibacterial activity of ethyl acetate against taken species of organisms

DISCUSSION

The present study deal with the Pharmacognostic, phytochemical, antioxidant, and antibacterial activity leaves of *Carica papaya*. The microscopic study of leaf showed the presence of epidermis (upper and lower) lower epidermis consist numerous stomata but not in upper epidermis. Beneath epidermis a layer of palisade cells and spongy cells are present. Spongy cells consists rosette crystal. Pith is hollow. The epidermal layer of leaf shows the presence of anomocytic type of stomata.

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one which is more predominate to other. It may also help in searching bioactive agents that could be used in the synthesis of very useful drugs. The phytochemical test result showed that the hexane extract indicate the presence of alkaloid, tannin, carbohydrate, flavonoid, phenol, protein and absence of glycoside, saponin, terpenoid, cardiac glycoside and anthraquinone glycoside. The ethyl acetate extract indicate the presence of alkaloid, tannin, carbohydrate, flavonoid, phenol, protein and absence of saponin, terpenoid, glycoside, cardiacglycoside, anthraquinone glycoside. The methanolic extract indicates the presence of Saponin, glycoside, tannin, terpenoid, carbohydrate, flavonoid, phenol, protein, cardiac glycoside and anthraquinone glycoside. The total phenolic content (TPC) and the total flavonoid content (TFC) of the extracts were expressed as milligram of Gallic Acid Equivalent per gram of extracts i. e. mg GAE/g extract and milligram of Quercetin Equivalent per gram of extract i. e. mg QE/g extract respectively. The amount of total phenolics and flavonoids of *Carica papaya* was measured by Folin-ciocalteau and colorimetric assay, the values were varied in different extracts and ranged from 9.43 in hexane, 19.93 in ethyl acetate and 41.45 in methanol mg GAE/g of extract in total phenol and from 29.21 in ethyl acetate, 29.73 in hexane and 32.42 in methanol, mg QE/g of extract for total flavonoid. The sequence of antioxidant activity of *Carica papaya* leaves extracts were; methanol (with IC50 value 50.056µg/ml) >ethyl acetate (IC50 value 54.041µg/ml) > hexane (IC50 value 175.12µg/ml). The antibacterial screening of hot extracted hexane and methanolic extracts of *Carica papaya* was found to be non-indicative to the tested stamp of microorganisms. Whereas the hot extracted ethyl acetat extract of *Carica papaya* in 100mg/ml concentration was found to have indicative antibacterial activity only in *Salmonella*

typhi(with ZOI 19) but non-indicative to *Staphylococcus aureus*, *Bacillus cereus* and *E.coli.*

CONCLUSION

The result of the study revealed that the methanolic extract consist large numbers of phytochemicals i.e. alkaloid, glycoside, tannin, terpinoid, saponin, cardiac glycoside, anthraquinone glycoside, flavonoid, phenol and protein. The study concluded that the methanolic extract of *Carica papaya* has significant antioxidant activity with highest TPC (41.458 mg GAE/g) and TFC (32.420 mg QE/g). Antibacterial activities of hexane and methanol extract of *Carica papaya* against the tested stamps of microorganisms were found to be non-indicative. Only the antibacterial activity of ethyl acetate extract *Carica papaya* was found to indicative against *Salmonella typhi* (with ZOI 19mm).

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REFERENCES

1. Azaizeh, H., et al., *Traditional Arabic and Islamic Medicine (TAIM) now joins TCM CAM, Kampo and Ayurveda.* Evidence-based complementary and alternative medicine: eCAM., 2008; 5(3): 363.
2. Balick, M.J. and P.A. Cox, *Plants, people, and culture: the science of ethnobotany*. 1996: Scientific American Library.
3. Shariff, Z.U., *Modern herbal therapy for common ailments*. 2001: Spectrum Books.
4. Mello, V.J., et al., *The gastric ulcer protective and healing role of cysteine proteinases from Carica candamarcensis.* Phytomedicine, 2008; 15(4): 237-244.
5. Munoz, V., et al., *The search for natural bioactive compounds through a multidisciplinary approach in Bolivia. Part II. Antimalarial activity of some plants used by Mosetene indians.* Journal of ethnopharmacology, 2000; 69(2): 139-155.
6. El Moussaoui, A., et al., *Revisiting the enzymes stored in the laticifers of Carica papaya in the context of their possible participation in the plant defence mechanism.* Cellular and Molecular Life Sciences CMLS, 2001; 58(4): 556-570.
7. Zunjar, V., et al., *Pharmacognostic, physicochemical and phytochemical studies on Carica papaya Linn. leaves.* Pharmacognosy Journal, 2011; 3(20): 5-8.
8. Savithramma, N., M.L. Rao, and D. Suhrulatha, *Screening of medicinal plants for secondary metabolites.* Middle-East Journal of Scientific Research, 2011; 8(3): 579-584.
9. Gautam, A., et al., *Identification, evaluation and standardization of herbal drugs: a review.* Der Pharmacia Lettre, 2010; 2(6): 302-315.
10. Singleton, V.L., R. Orthofer, and R.M. Lamuela-Raventós, *[14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent.* Methods in enzymology, 1999; 299: 152-178.
11. Quettier-Deleu, C., et al., *Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour.* Journal of ethnopharmacology, 2000; 72(1): 35-42.
12. Subedi, A., et al., *Antioxidant and antibacterial activity of methanolic extract of Machilus odoratissima.* Kathmandu University Journal of Science, engineering and technology, 2012; 8(1): 73-80.
13. Satish, S., M. Raghavendra, and K. Raveesha, *Evaluation of the antibacterial potential of some plants against human pathogenic bacteria.* Advances in biological research, 2008; 2(3-4): 44-48.
14. Joshi, B., et al., *Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem).* Journal of Microbiology and Antimicrobials, 2011; 3(1): 1-7.