CHEMICAL ANALYSIS OF LEAVES AND FRUITS EXTRACT OF *CARICAPAPAYA* AND ASSESSMENTS OF THE IN *VITRO* BIO ACTIVITIES

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ABSTRACTS.

Carica papaya, also simply known as papaya or pawpaw. A fruit-bearing tree growing in different parts of the world. Fruit , leaves and seeds of papaya are being use in folk medicines since centuries . Fruit contains secondary metabolites those help to minimize many human disorders. Antioxidants and antimicrobial effects of leaves and fruit extracts of *Carica papaya* were evaluated in current experimental work. Results indicates that leaves and fruit extracts have provided remarkable antioxidants activities when DPPH, H_2O_2, ABTS and reducing power assays were carried and compared with standards The leaves and fruit extracts has provide prominent growth of inhibition against four bacterial and fungal strains and noteworthy minimum inhibitory concentration ethanolic leaves extracts was observed. Analysis of various extracts for phytochemicals with UV/Visible spectrophotometer and high performance liquid chromatography revealed presence of significant amount of total phenols, flavonoids and other secondary metabolites. Which indicates that these extracts might be good source of pharmaceutical materials required by industries for preparation of various drugs for human health.

Key words: *Carica papaya*, Antioxidants, Antimicrobial, Secondary metabolites.

INTRODUCTION

Although human research is lacking, many papaya leaf preparations, such as teas, extracts, tablets, and juices, are often used to treat illnesses and promote health in numerous ways. Papaya leaf contains unique plant compounds that have demonstrated broad pharmacological potential in test-tube and animal studies.

Therefore, to increase proper judgmentabout use of plants for health purpose information regarding their properties, safety and efficiency must be disseminate to human population (Ellof, 1998).According to information available in literature the interest of consumers is nowfocused to use products having natural antioxidants as compared to synthetic as well as to avoid the use of drugs having carcinogenic side effects after consumption (Velioglu *et al*., 1998).Many medicinal plants contains large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing oxidative reaction.

The Plant source nutrients acquired substantial antioxidant capabilitythat islinked with lesser mortality rates of many human diseases (Anderson *et al*.,2001).

According to Chen (1992)battle of synthetic antibiotics drugs against microorganism is not successful as many bacteria have genetic ability to show resistanceforthese drugs after couple of years and due to this new scenario of hospitalized patients got suppressed immunity condition ,Consequently, new infections can occur in hospitalized patients those could worsen the condition ,resulting in high mortality rates (Chem *et al*., 2017).

The fruits and vegetables containsseveral compounds thoseareconsidered as anti-cytotoxic ,antigen toxicand are able to reduce incidence of tumors .Therefore, understanding about health benefits and/or potential toxicity of these plants is important.

The common and easy method to detect cytotoxicity of plant materials is the brine shrimp assay. This bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts etc. (Pisutthanan *et al*., 2004; Mclaughlin *et al*., 1991)

Carica papaya L.,*belongs* to family Caricaceaeis excessively distributed throughout the tropics and subtropicscountries.It is a berry like fruit grow from ovary that is superior and its placentation is parietal (Kochhar, 1986). Theleaves andfruits of papayacontainedphenols, flavonoids, carotenoids, essential vitamins and minerals (Rahmat *et al*., 2012).The Carica latex contained chitinase, glutaminly cyclase and cysteine endopeptidases(Papain chymopapain and caricain). Linalool in fruit pulp and alkaloids like carpaine, pseduocarpaine, dehydrocarpaine I and II (Lim, 2012). Different parts of the papaya plants including fruits, leaves, stems, seeds and roots have long been used as ingredients in alternative medicines. The antioxidant properties of *C. Papaya* fruits is very useful and it very helpful in defending the body against reactive oxygen species. These destructive products areproduced as a result oflipid oxidation in the human body. Reactive oxygen species (ROS) are presently considered as source for severalhuman infections (Repettoand Llesuy, 2002; Rahmat et *al*., 2004). Many tropical and subtropical countries are engulfed by dengue infections which is caused by viruses belonging to the Flaviviridae family. Many reports are available in literature indicates thatpeople effected by dengue fever are restored by use of Papaya leaf extracts. However,scientific prove about use of Papaya leaf extracts against dengue feveris still not available.Therefore keeping in view thebeneficial effects of leaves and fruit of *C.papaya* the present study was designed toassess antioxidants, antimicrobial and cytotoxiceffects of leaves and fruit extracts of *Carica Papaya* (Rahmat*et al*., 2004).Many tropical and subtropical countries are engulfed by dengue infections which is caused by viruses belonging to the Flaviviridae family. Many reports are available in literature indicates thatpeople effected by dengue fever are restored by use of Papaya leaf extracts. However,scientific prove about use of Papaya leaf extracts against dengue feveris still not available.Therefore keeping in view thebeneficial effects of leavesand fruit of*C.papaya*the present study was designed to assess antioxidants, antimicrobial and cytotoxic effects of leaves and fruit extracts of *C. papaya.*

MATERIALS AND METHODS

Collection and preparation of samples.

Leaves and fruit samples of *C. papaya* were collected from different areas of Rawalpindi, identified by expert and registered specimen(voucher no, 142) for future reference. The leaves and fruit samples were shadow dried, then sun drying followed by oven drying at 60ºC. The dried samples were ground into powder form (80 meshes) and saved in fine plastic bags for further uses.

Phytochemical analysis

Total 200 g of fruit and leaves samples of *C. papaya* were separately placed in 400 ml round bottle flask. The samples weremacerated andextracted with n-hexane,chloroform, methanoland ethanol. All procedures were developed at room temperature. The leaves and fruit extracts were used for qualitative andquantitative analysis of secondary metabolites by using methods reported by Sofidiya*et al.*(2006), Harbone (1973)and Trease and Evans (1983).Total phenolic contents were evaluated from various extracts of *C. papaya* (Kimet *al*., 2003).Whereas flavonoids contents of leaves and fruit extracts werequantified by using method reported by Hussain et *al*. (2012).

Chromatographic analysis

The ethanolic fruit extracts which has shown higher amountsof totalflavonoids was further quantified with higher performance liquid chromatography by using isocratic elution , withmethanol andwater mixture (70:30) with flow rate of1 ml/min, retention time of 20 minand by providing 20µl sampleto C18 column at 30ºCand amount of quercetin (flavonoid)was quantified byusingUV/visible , detector at368 nm wavelength.

Antioxidant activities of leaves and fruit extracts of C. papaya

The capacity of the both leaves and fruitsextracts to reduce Fe+3 ions into Fe+2 ions was assessed as describedbyYildirim *et al.* (2001).Whereas scavenging ability was determined by using DPPH assay, reported by Moon and Shibamoto(2009). ABTS assay was conducted to carry out free radical scavenging capability of extracts (Ashafa *et al*., 2010).The capacity of extracts to scavenge $H_2O_2$was assessed on the basis of procedure published by Ruch *et al*. (1989).

Antibacterial and antifungal activities of extracts

Plant samples were screened to determined antibacterial potential by using agar well diffusion assay against four bacterial strain , *Staphylococcus aureus*(ATCC 6538), *Escherichia Coli* (ATCC15224), *Klebsiella pneumonia* (MTCC618) and *Bacillus subtilis* (ATCC6633). Standard antibiotics (Cefiximeand Roxithromycin) wereused for comparison andabsorbance was determined at 420 nm by using spectrophotometer (Boyed, 1980). Theminimum inhibitory concentration was estimated on the basis of lowest concentration of the extracts that blocked the further progress of bacteria after 24 hours of incubation period. The antifungal activity of *C. papaya* extractswere estimated by usingagar tube dilution methodagainstfourstrains of fungus(*Aspergillus niger* ,0198;*Aspergillus flavus* ,0064; *Aspergillus fumigates* ,66 and*Fusarium solani*, 0291)as reported earlierthroughEttebong and Nwafor (2009)

STATISTICAL ANALYSIS

The values were represented as means± SD after statistical analysis

RESULTS AND DISCUSSION

Phytochemical analysis is prerequisite for investigation of pharmacological potential of any plant extracts and its fractions.According to resultsobtained in this studyalkaloids, flavonoids, phenols, tannins, cardiac glycosides, saponins and terpeneswere found in fruit and leavesextractsof *C.papaya* (Table 1).

The quantification of bioactive compounds like phenolics and flavnonids from fruit and leaves extracts were carried (Table 2).

Table 1. Assessment of various phytochemicals fromleaves andfruit extracts of *C.papaya*

Extracts	Alkaloids	Flavonoids	Polyphenols	Tannins	Cardiac glycosides	Saponins	Terpenes
Ethanolicleaves extracts	++	+	+	++	+	-	+
Ethanolicfruit extracts	+	+++	++	+	++	+	++

+; present ; - absent

Table 2. Assessment of Flavonoidsand Phenoliccontents of leaves andfruit extracts of*C. papaya*

Extracts	**Total Flavonoids (mg rutin equivalent/g)**	**Total phenols (mg GAE/g**	**Yield of extracts (%)**
Ethanolicleaves extracts	47.12 ± 2.15 *	47.22 ± 2.14*	4.6 ± 0.7
Methanolic leavesextract	23.11 ± 0.51	25.63 ± 1.32	2.5 ± 0.4
n-hexane leaves extracts	15.15± 1.36	18.54± 1.71	2.4 ± 0.5
Ethanolic fruit extract	46.27± 1.23*	46.71 ± 1.32*	1.3 ± 0.6
Methanolic fruit extracts	21.15 ± 4.1	18.54 ± 2.93	1.5 ±0.7
n-hexane fruit extracts	15.110 ± 0.51	15.66 ± 1.3	2.4± 0.4

Values are represent as mean +SD (n=3); *significantly higher value ($p<0.05$)

Analysis of various extracts of *C. papaya* with HPLC revealed thathigher quantity of quercetin was present in ethanolic leaves extracts as compared ethanolic fruit extracts. Canini *et al.* (2007) and (Yap *et al*., 2020) found quercetinin *C. papaya* samplesbut quantity reported by them were lower as compared toquantity of quercetin found incurrentstudy (Fig 1 and 2).

To check the antioxidant potential of leaves and fruit extracts of *C. papaya* , fivedifferent assays i.e. DPPH, H_2O_2 , ABTS Phosphomolybdate and reducing powerassays were performed and values obtained were compared with ascorbic acid and Rutin used as standard in this experiment. Although all solvents extracts have shown a remarkable antioxidant potential, but antioxidant potential shown by ethanolic leaves and fruit extracts was more prominent has as compared to other solvents extracts. However, in some cases the values were lower than positive control (ascorbic acid) and standard rutin (Table 3).

The ethanol leaves and fruit extracts has shown active inhibitory potential against*S. aureus* , *E.coli*, *K. pneumonia* and *B. subtilis* strains as compared to all other extracts , cefixime and DMSO, however, lower zones of inhibition were observed as compared to roxithromycin (standard) (Table 4).

Minimum inhibitory concentration indicates significant antimicrobial potential of extracts and data obtained, through the determination of MIC of various extracts and antibiotics are presented in table 5.

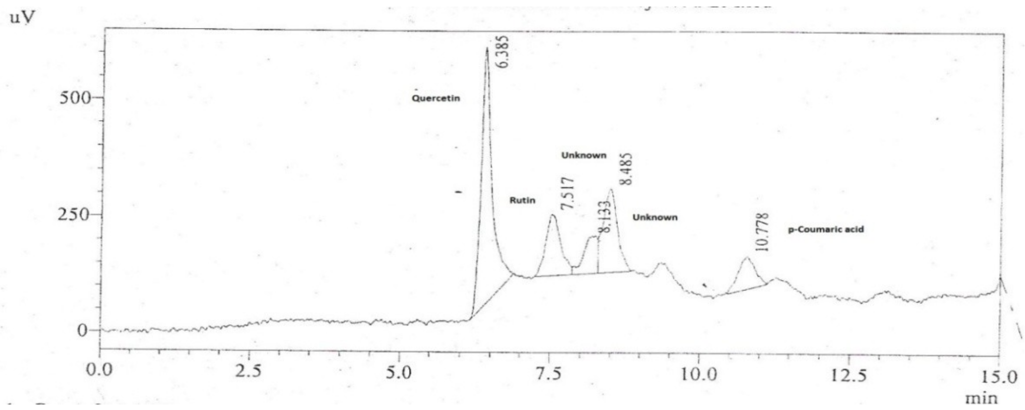

Figure 1.HPLC analysis of ethanolic fruit extractsof *C. papaya*

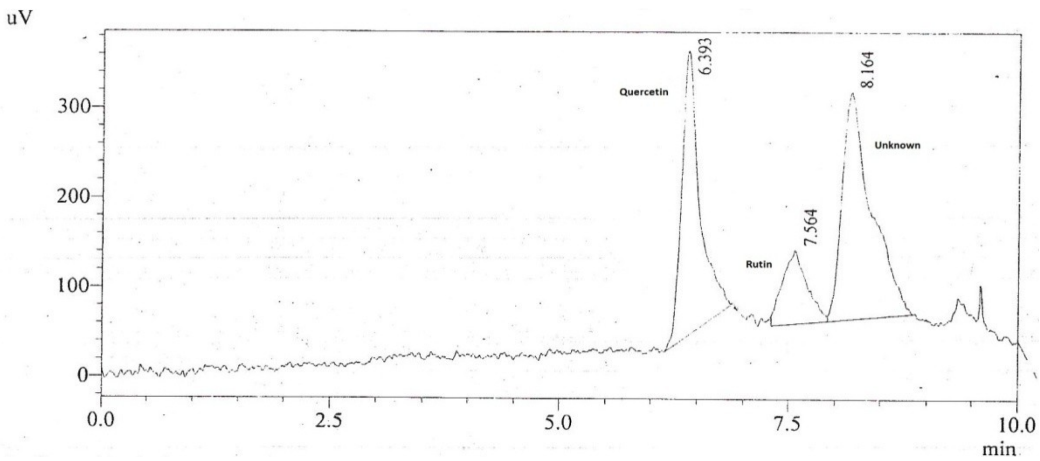

Figure 2.HPLC analysis of ethanolic leaves extracts of *C. papaya*

Table 3. Antioxidant potential of various extractsof *C. papaya* at concentration of 100 µg/ml andIC 50 values (µg/ml) of radical scavenging when absorbance was measured at 700 nm

Extracts	**DPPH**	H_2O_2	ABTS	Reducing Power assays	Ascorbic acid	Rutin
Leaves extracts						
Ethanol	0.114±0.01	2.132±0.05	1.323±0.08	0.135±0.01	1.214±0.81	1.772±1.83
Methanol	0.251±0.03	2.251± 0.04	2.252±0.07	0.154±0.01	1.252 ±0.34	2.424±0.84
n-hexane	0.311±0.01	2.653± 0.05	2.656±0.07	0.532±0.06	1.545 ±0.32	1.551±0.75

Chloroform	0.023±0.01	1.924±0.71	7.655± 0.15	0.163±0.01	1.825±0.45	1.662±0.52
Aqueous	3.081±0.01	8.132±0.26	9.134±0.26	0.184±0.05	1.751±0.52	1.874±0.35
Fruit extracts	0.275±0.03	2.215±0.04	2.353±0.07	0.156±0.01	1.432±0.33	1.773±0.81
Ethanol	0.123±0.00	0.851±0.01	0.382±0.19	0.113±0.01	0.435±0.82	0.956±0.25
Methanol	0.254±0.03	2.352±0.04	2.252±0.07	0.135±0.01	1.225 ±0.34	1.434±0.83
n-hexane	0.125±0.01	1.193±0.81	1.911±0.52	0.196±0.05	1.362±0.55	1.125±0.35
Chloroform	0.223±0.01	1.254±0.93	1.653±0.74	0.091±0.06	1.554±0.36	1.435±0.54
Aqueous	0.318±0.01	1.435±0.75	1.864±0.43	0.182±0.01	1.635 ±0.72	2.254±0.36

Results are Means ± SD, (n = 3).

Table 4 Antibacterial activities of various extracts *C. papaya*; Zone of inhibition in mm.

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime	DMSO
Leaves extracts							
Ethanol	24.6±0.6	25.4±0.9	29.5±0.3	25.6±0.4	24.3±0.9	13.5±0.3	0.0
Methanol	21.6±0.8	22.6±0.6	26.6±0.7	24.6±0.6	22.6±0.7	11.6±0.5	0.0
n-hexane	17.2 ±0.5	17.3±0.8	17.6±0.4	16.5±0.7	12.3±0.9	11.4±0.8	0.0
Chloroform	18.3±0.6	19.2±0.3	14.2±0.4	15.8±0.5	13.6±0.8	12.7±0.9	0.0
Aqueous	12.4±0.8	15.6±0.6	11.3±0.5	11.6±0.3	10.2±0.7	9.4±0.8	0.0
Fruit extracts							
Ethanol	23.5±0.3	24.8±0.4	23.5±0.7	16.8±0.6	14.3±0.7	15.8±0.9	0.0

Methanol	22.6±0.9	23.5±0.7	22.6±0.8	13.4±0.5	11.9±0.3	12.6±0.5	0.0
n-hexane	19.6±0.5	19.2±0.9	19.2±0.6	14.5±0.5	14.5±0.7	15.3±0.8	0.0
Chloroform	13.4±0.4	15.7±0.8	21.6±0.5	15.7±0.9	13.6±0.7	14.8±0.5	0.0
Aqueous	11.5±0.7	13.2±0.7	13.5±0.4	11.2±0.7	9.7 ±0.8	8.2±0.3	0.0

Results mean± S D after triplicate analysis (n=3).

Table 5. Minimum inhibitory concentration (µg/ml) of *C. papaya*extracts for various bacterial strains.

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime	DMSO
Leaves extracts							
Ethanol	0.1±0.6	1.2±0.7	0.8±0.5	1.4±0.6	1.3±0.8	1.5±0.5	0.0
Methanol	0.5±0.2	2.2±0.3	1.1±0.3	2.1± 0.4	1.9±0.5	2.2 ±0.6	0.0
n-hexane	1.7 ±0.1	1.6±0.9	0.7±0.4	1.6±0.7	1.2 ±0.3	1.1±0.8	0.0
Chloroform	1.8±0.9	1.9±0.5	1.2.2±0.4	1.5±0.5	1.3±0.5	1.2±0.5	0.0
Aqueous	2.9±0.3	2.6±0.7	2.7±0.5	2.6±0.3	2.2±0.6	2.4±0.3	0.0
Fruit extracts							
Ethanol	0.5±0.6	0.8±0.1	0.7±0.9	1.1±0.4	0.2±0.7	0.8±0.3	0.0
Methanol	0.8 ±0.2	1.3± 0.4	1.1±0.2	1.6±0.6	0.7 ±0.1	1.4 ±0.1	0.0
n-hexane	1.6±0.5	1.9±0.8	1.9±0.5	1.5±0.5	1.5±0.9	1.1±0.8	0.0
Chloroform	1.4±0.4	1.5±0.9	1.6±0.7	1.4±0.6	1.6±0.4	0.4±0.6	0.0
Aqueous	1.5±0.7	1.3±0.7	1.8±0.4	1.9±0.3	1.7 ±0.8	2.2±0.3	0.0

Results are Means ± SD, (n = 3)

Data in table 6 represents the antifungal activity of plants extracts , according to results higher fungal activity was represent by ethanolic leaves extracts (25.8±0.5mm) followed by ethanolic fruit extracts (24.5 ±0.1mm) and methanolic leaves extracts (23.4± 0.3) . While lowest activity was shown by aqueous leaves extract (10.5±0.6 mm). Extracts prepared in organic solvents has shown reliable antifungal activities and these results are comparable to results of such extracts earlier reported by Fawole *et al.* (2008), Parekh and Chanda, (2007) and Wijesooriya *et al*., 2019) found after experiment that water extracts showed no/poor fungus toxicity than organic solvents, which is also indicated in present study (Table 6).

Table 6. Antifungal activities of variousextracts of *C. papaya*; Zone of inhibition in mm.

Extracts	*Aspergillus Niger*	*Fusarium salani*	*Aspergillus Flavous*	*Aspergillus Fumigates*	Terbinafine	DMSO
Leaves extracts						
Ethanol	25.8±0.5	24.8±0.4	18.3±0.3	21.5±0.4	18.4±0.3	0.0
Methanol	23.4 ±0.3	22.5±0.7	16.5 ±0.6	19.5 ±0.2	16.3±0.8	0.0
n-hexane	18.3 ±0.5	17.2±0.5	17.8±0.4	15.4±0.8	15.3±0.5	0.0
Chloroform	16.3±0.4	18.2±0.3	16.3±0.4	15.8±0.5	14.6±0.3	0.0
Aqueous	11.4±0.2	9.6±0.3	11.6±0.2	11.6±0.3	10.1±0.6	0.0
Fruit extracts						
Ethanol	24.5±0.1	19.8±0.3	21.1±0.5	19.6±0.3	16.3±0.6	0.0
Methanol	22.6±0.5	18.2±0.7	19.3±0.8	16.5±0.5	14.8±0.4	0.0
n-hexane	18.6±0.3	17.2±0.9	17.2±0.9	18.5±0.6	15.5±0.4	0.0
Chloroform	16.4±0.2	16.1±0.9	16.6±0.3	15.7±0.9	14.6±0.7	0.0
Aqueous	10.5±0.6	11.2±0.4	11.5±0.4	9.2±0.5	9.7 ±0.3	0.0

DISCUSSION

Phytochemicals based on plants, vegetables and fruits discrete bioactivities towards human metabolism are being widely examined for their ability to promote health benefits. Based on traditional knowledge about use of *C.papaya*, the study was designed to provide scientific proof about safe and beneficial consumption of leaves and fruit of this plant.In this experimental work the ethanolic leaves and fruit extracts of*C. papaya* has provided higher amount of total phenol and flavonoids contents as compared to other extracts analyzed(Table 2, Figures 1 and 2).Which indicates worth of leaves and fruit of *C. papaya* and suggests its usefor human health. The results of phytonutrients obtained in present study were comparable with results reported earlier by other authors including Kim *et al*. (2003). Phenolic compounds are secondary metabolites naturally present in all plants in wider range, possess free radical scavenging activity and considered as potential antioxidant agents having great impact on human health (Jayaprakash *et al*., 2001).Phenolic compounds are very important antioxidants, having various application like reducing agents, hydrogen donors, and free radical scavengers, quenchers of oxygen and as cell saviors. The concentration level of flavonoids found in *C. papaya* during present study was higher than reported by Omotade *et al*. (2011). Flavonoids possess antioxidants, antiviral, anti-fungal and antibacterial activities but alsoprotect liver and stomach from different disorders (Okeniyi*et al*., 2007). Quercertin is a flavonoids found in leaves and fruit extracts of *C. papaya* (Table 2,Figs 1 and 2). Prevents human body from releasing histamines that cause an allergic response in the body. Quercetin also prevents plaque buildup in arteries (atherosclerosis)that can lead to heart attack, stroke, and obesity (Chirumbolo, 2012).

It was reported by Aqil *et al*. (2006) that plant extracts containing isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins always have good potential of antioxidant activities.

Analysis of *C. papaya* extracts with HPLC revealed the presence of reliable quantity of quercetin (Figs. 1and 2)and probably this flavonoid along with other secondary metabolites was responsible for majority of biological activities of theseextractsas reported by Rathee *et al*. (2008).Whereas Devasagayam *et al*. (2004) found that drugs having good potential of antioxidants are useful for the treatments of complex infections of human population like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer etc.,

Allextracts showed a remarkable antibacterial potential against both Grams +ve and Gram –Vebacterial strains as well as fungal strains which indicatesthat plants extracts composed of antimicrobial components (flavonoids etc.,)that can be used to treat infectious disorders caused by the severe resistant pathogenic microorganisms. The current findings support the results reported by other authors with slight variations (Ettebong, and Nwafor, 2009).The uses of plant based drugsagainst infectious diseases around the world have long history, that have strong potential for effective treatments ofantibiotic-resistant infections , which is larger public concern and increasing globally (Fawole*et al*., 2009 :Parekh and Chanda, 2009).The results obtained in current study disclosed instability in the inhibitory concentrations of each extract for given bacteria. The lowest MIC was observed for ethanolextracts mightbe due to its purity or solubilityof plant materials in relevant solvents (Table 5). Results found in present study were comparable to results report by other authors including Guessan et *al*. (2007).

Results of brine shrimp lethality obtained in current study were comparable with results reported by Sandeep et *al*. (2009).The brine shrimp assayprovide important information about bioactivity of any plant extractthat might be correlated reasonably with cytotoxic and antitumor properties..

It is concluded that the *C. papaya* leaves and fruit extracts contained valuable bioactive secondary metabolites (Polyphenolsand Flavonoids). These extracts have shown good potential to scavenge free radicals, inhibited the growth of pathogenic bacterial and fungal strains. It is predicted that a use of appropriate doses of leaves and fruit extracts of *C. papaya* is helpful to improve many human disorders especially when antibiotics resistance or reduction in platelet levels in blood of any individual is in question. These pharmacological properties have make papaya fruit and leaves suitable for human consumption to minimize many of human aliments. Further research is needed to isolate lead compound that might be required by industries for preparation of drugs to cure various liver ailments.

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