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Research Article

Identification of Bioactive Constituents in Peel, Pulp of Prickly Custard Apple (*Annona muricata)* and its Antimicrobial Activity

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ABSTRACT
The present intend of the study to identify the bioactive components and its antimicrobial activity in the prickly custard apple *(Annona muricata)*, it is a common fruit in tropical Asia of annona species, is occasionally met under cultivation in Tamilnadu, Karnataka, Andharapradesh, Maharashtra, Assam and Andaman and Nicobar Islands. The fruit pulp, peel was used to extract by methanol and ethyl acetate and the methanolic extract was observed better effect compared to ethyl acetate extract, the peels and pulp were good source of bioactive components compare than seed samples. Hence the twenty-seven bio active compounds were identified in the peel, pulp and seed samples and the nine compound in peel nine in pulp and eleven compound found in seed specifically. Hence, single compound namely Dasycarpidan-1-methanol, acetate (ester) present in both peel and pulp were identified. These specific compounds mainly responsible for the antimicrobial activity and it is also observed the greatest antimicrobial effect against these particular microbes E. coli, S. aureus, Bacillus, Proteus, and Klebsiella species.

Keywords: Peel, Pulp, Seed and Methanol and Ethyl acetate.

INTRODUCTIODN

Prickly custard apple it is most tropical annona species, is occasionally met under cultivation in Tamilnadu, Karnataka, Andharapradesh, Maharashtra, Assam and Andaman and Nicobar Islands[1]. The prickly custard apple has with glossy, dark green leaves. It produces a large, heart shaped fruit that is 15 – 20 cm in diameter which is yellowish when ripped but whitish inside[2]. it belongs to the annonaceae family and is commonly known as Sour-sop. It is growing 5-6m in height it is one of the easily found plants used as traditional medicine[3]. Prickly custard skin is a dark green, but later yellowish-green and finally all yellow when over-ripe. The soft spines on the skin, the fully mature fruit are good or light greenish yellow. The ripe, mature fruit is soft to the touch; ripeness is better detected by touch than by colour. The outside of the fruit is thorny while the pulp is white and juicy with brownish seeds[4]. It is one of the most nutritious fruit in the tropical region. The white cottony pulp is very juicy, varying in flavour from acid to sweet and highly regarded because of its distinctive aroma and flavour. The flash or pulp that is creamy white in colour and has the texture of juicy floss interlaced with black seeds, the flavour was tropical, strong and pungent on entry but with a delicate ending a soothing creamy after taste[5]. This unique flavour of soursop have the role of some compounds like Esters were found to be the dominant flavour compounds regardless of the soursop's origin, with methyl (E)-2-hexenoate, methyl (E)-2- butenoate, methyl butanoate and methyl hexanoate being the four principal compounds involved the fruit development and other role[6]. In addition, health benefits of the crushed seeds are used to treat against internal and external parasites, head lice and worms. The use of complementary traditional medicine which includes herbal medicines in the treatment of various diseases has expanded rapidly in developing countries. Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited. There are several reasons why the use of medicinal plants should be studied: herbal remedies may have recognizable therapeutic effects[7]. Although, the use of medicinal plants provides an indication of illness and its treatment that may conflict with beliefs of workers in the formal healthcare system[8]. The juice of the fruits is taken orally as herb remedy of arthritis, haematuria and liver ailments, pulverizing the Annona seed and mixing it with soap and water is used as effective spray against caterpillar[9]. Generally, the fruit and fruit juice are taken to eliminate worms and parasites, cool fever, increase mother's milk after child birth, and is as an astringent for diarrhoea and dysentery[10]. The crushed seeds are used against internal and external parasites, head lice and warms[9]. Hence, in Jamaica, Haiti and the west indies, the fruit and or fruit juice is used for fever, parasites and

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diarrhea[11]. Recent studies concentrate only in leaves and the leaves medicinal properties but no more studies available in relate with the seed and other part of the annona fruit, basis of the previous studies the present study focus on the other part of annona fruit were select and the study revealed that the antimicrobial activity. This fruit have been focus to several studies carried out around the world, reporting in their nutritional values, and data especially in relation to the evaluation of antimicrobial activity. Based on the previous reports the present study aims to identify the bioactive compounds in Annona muricata peel and pulp extract and their effect of the antimicrobial activity.

methodology

collection and processing of samples

Prickly custard apple fruit was collected from Jalagandapuram village. The sample was collected by hand picking. The collected fruit prickly custard apple was washed with fresh water to separate contaminates such as adhering impurities, sand particles and dust. Then cut the fruit separated the peel, pulp and seed. The peel, pulp and seed were allowed to shadow dry in a room temperature for four days. The dried peel, pulp and seed were taken out after four days. The dried peel, pulp and seed samples were uniform make it in powder using mixer grinder machine. The powder was stored at until further analysis.

aqueous extraction of prepared samples peel and pulp

The powder obtained was extracted with distilled water. 5g of powered sample added 100ml of distilled water and boiled to 100 C for about 15 min. then the resulting curd were filtered through normal filter paper and stored in refrigerator.

estimation of total phenol content

The determination of total phenol content in each sample (12 no samples) was estimated by Folin-Ciocalteu (F-C) assay. Briefly a volume of 100µl of sample was added to 2ml micro centrifuge tube followed by 860µl distilled water, 50µl F-C reagents, mixed and allowed to react for 5 min before adding 100µl 20% Na_2CO_3, 890µl distilled water. All the added solutions were mixed and allowed to stand 60 min at room temperature than measured absorbance at 725nm.The blank was prepared in similar manner without sample and standard. Calibration curve was plotted using Gallic acid as standard (10, 20, 40, 60, 80,100µg/ml). The results were expressed as milligram of Gallic acid equivalents (GAE) per gram of extract.

estimation of total flavanoid content

Total flavanoids were estimated by Aluminium chloride colorimetric assay. 1 ml aliquot of appropriately diluted each sample or standard solution of quercetin (10, 20, 40, 60, 80 and 100µg/ml) was mixed with 50µL of $NaNO_2$ in 2ml micro centrifuge tube. After 6 min, 50µL of a 10% Aluminium chloride solution was added and allowed to stand for 6 min, and then 50µl 1M potassium acetate solution was added to the mixture. Distilled water was added to bring the final volume to 2ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm against prepared blank. Blank was prepared in the same above manner omitting sample/standard. All values were expressed as milligrams of quercetin equivalent per 1g of sample.

gc-ms analysis

gas chromatography

An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2 minutes, then ramp at 20° C per minute to 300°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

mass spectrometry

A JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-20001 software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

mass spectrometry library search

Identification of the components of the purified compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software.

anti-microbial activity

Agar well diffusion technique

The extract was tested for antibacterial activity by standard agar well-diffusion method against bacteria. The pure cultures of bacterial pathogens were sub cultured on nutrient broth. 20ml of Muller Hinton agar were poured into the Petri plates. Wells of 6 mm diameter were made on agar medium using gel puncture. Culture was swabbed uniformly using sterile cotton swabs, and then 100µl of plant extract solution was loaded into the wells. After incubation at 37°C for 24 hours, the different levels of zone of inhibition were measured[12].

RESULTS

The GCMS analysis showed that the compound fed between 09 to 25 minutes' figure 1,2 and 3 show the mass spectrum of compound identified in prickly custard apple peel pulp respectively. There are twenty-seven compounds were identified in the peel and pulp of prickly custard and the identified compound shown in the (Table.1). Sixteen were noted to be aliphatic compounds and fifteen were aromatic compounds and the eight compounds are identified from peel that are namely, Dasycarpidan-1-methanol, acetate(ester), 6-Octadecenoic acid, (Z), Eicosanoic acid, Ethanol,2-(9-octadecenyloxy)., (Z), Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate, Dasycarpidan-1-methanol, acetate(ester), 4-Hexyl-1-(7-ethoxycarbonylheptyl) bicycle (4.4.0) deca-2,5,7-triene, Ethonol, 2, (9-octadecenyloxy)-, (Z). There are eight compounds were identified from pulp namely 1,2,12-Nonadecatriene.5,14-diol, Gibb.3.ene.1,10-dicarboxylic

Table 1: Bioactive compounds identified in the peel and pulp of prickly custard apple (*Annona muricata*).

S. No.				
1.	14.96	Dasycarpidan-1-methanol,acetate(ester)	+	-
2.	10.31	6-Octadecenoic acid,(Z).	+	-
3.	11.33	Eicosanoic acid	+	-
4.	11.8	Ethanol,2-(9-octadecenyloxy).,(Z)	+	-
5	12.52	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate.	+	-
6.	12.68	Dasycarpidan-1-methanol,acetate(ester)	+	+
7.	14.12	4-Hexyl-1-(7-methoxycarbonylheptyl)bicycle(4.4.0)deca-2,5,7-triene	+	-
8.	14.96	Ethonol,2,(9-octadecenyloxy)-,(Z).	+	-
9.	12.55	1,2,12-NONADECATRIENE.5,14-diol	-	+
10.	12.65	Gibb.3.ene.1,10-dicarboxylic acid,2,4a, dihydroxy-1-methyl-8-methylene. 1,4a-lactone,10-methyl ester.	-	+
11.	12.83	Corynan-17-ol,18,19-didehydro-10-methoxy	-	+
12.	13.33	5,8,11-heptadectrien-1-ol	-	+
13.	13.62	Chromon,5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl	-	+
14.	14.73	Dasycapidan-1-Methanol,acetate(ester)	+	+
15.	16.22	Isomenthone	-	+
16.	11.38	Methyl 2-cholorohexadecanoate	-	+
17.	12.63	1H-Pyrrolo[2,3-c]pyridine-3-propanicacid,5(4H)-oxo-6,7-dihydro.Methyl ester	-	-
18.	16.2	Pregn-9(11)-en-20-one,3,6-bis[(methylthio)methoxy].	-	-
19.	10.55	Arabintol,pentaacetate	-	-
20.	11.38	Cyclopropanebutanoicacid,2-[(2-[(2[(2-pentylcyclopropyl)methyl)cyclopropyl)methyl ester	-	-
21.	11.63	5-Thio-D-glucose	-	-
22.	12.5	[1,1'-Bicyclopropyl]-2-octanoic acid,2'-hexyl-,methyl ester	-	-
23.	12.63	1H-Pyrrolo[2,3-c]pyridine.3-propanic acid,5(5H)-oxo-6,7-dihydro., methyl ester	-	-
24.	13.33	10-heptadecen.8-yonic acid, methyl ester,(E).	-	-
25.	13.58	Arabinitol,pentaacetate	-	-
26.	14.72	Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl).	-	-
27.	18.25	Triamcinolone acetonide	-	-

Table 2: Absorbance of Gallic acid standard

S. No.	Concentration µg/ml	Absorbance
1	10	0.026
2	20	0.068
3	40	0.149
4	60	0.237
5	80	0.319
6	100	0.398

Table 3: Absorbance of quercetin standard

S. No.	Concentration µg/ml	Absorbance
1	100	0.009
2	200	0.106
3	400	0.334
4	600	0.567
5	800	0.795
6	1000	1.059

acid,2,4a,dihydroxy-1-methyl-8-methylene, 1,4a-lactone,10-methyl ester, Corynan-17-ol,18,19-didehydro-10-methoxy, 5,8,11-heptadectrien-1-ol , Chromon,5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl, Dasycapidan-1-Methanol,acetate(ester), Isomenthone, Methyl 2-cholorohexadecanoate Interestingly, one of the compound present in both peel and pulp namely Dasycapidan-1-Methanol,acetate(ester) and Seven compound unique to pulp and seven compound unique to peel and eleven compound unique to seed samples. Based on the present investigation the single compound may act as a better stimulator for the antioxidant activity.

total phenol and flavanoid content

The total phenol and flavanoid content that is Gallic acid was calculated from prickly custard apple peel and pulp, totally the phenolic content in annona peel 261mg/gm, Pulp= 227mg/gm respectively. In the case of Gallic acid higher in peel when compared to that of pulp. The Gallic acid was used as standard and concentration of the standard 10 to 100µg/ml and the absorbance are 0.029, 0.068, 0.149, 0.257, 0.319, and 0.396 respectively it was shown in (Table.2 and Graph 1). The total quercetin was calculated from prickly custard apple (peel 261mg/gm, Pulp= 227mg/gm) interestingly, it was higher than the

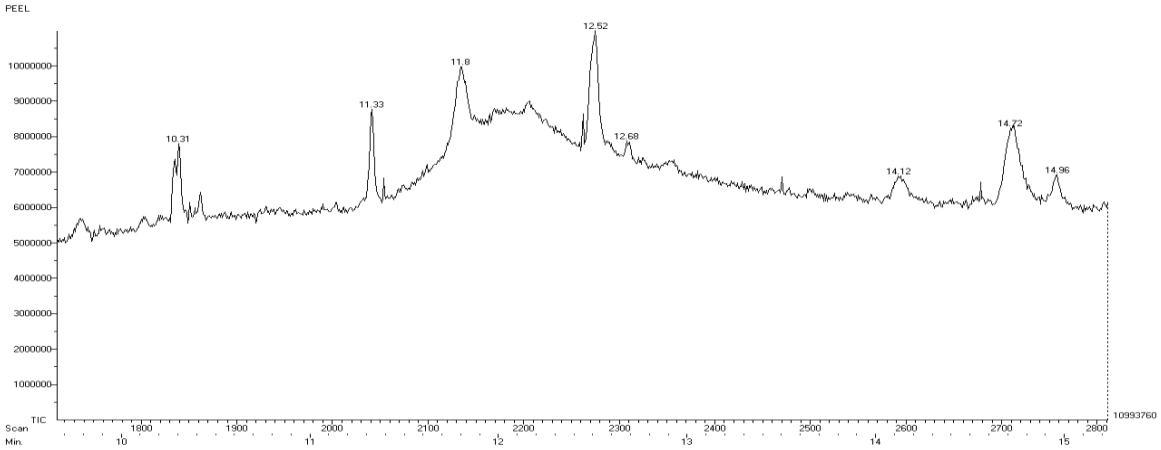


Figure 1: GC-MS profile of prickly custard apple peel sample.

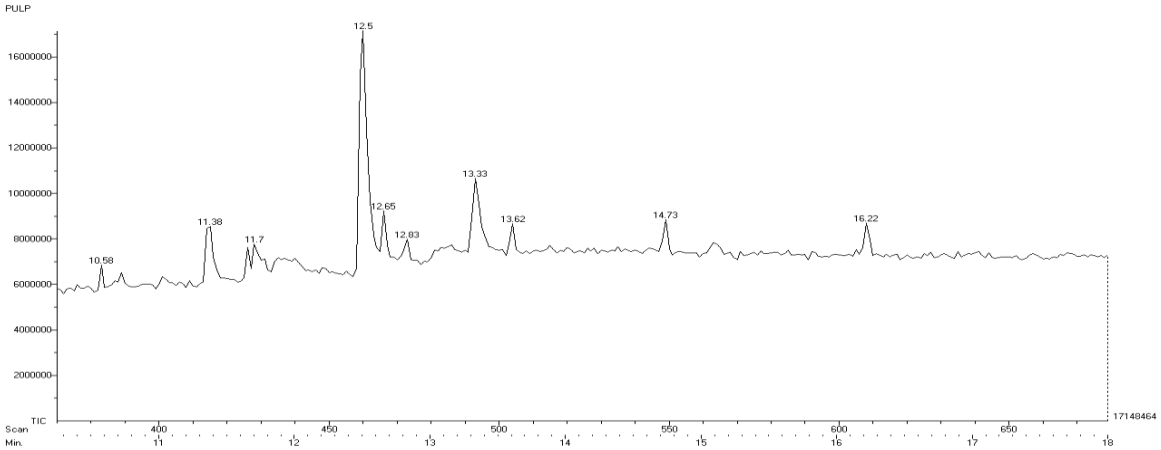


Figure 2: GC-MS profile of prickly custard apple pulp sample.

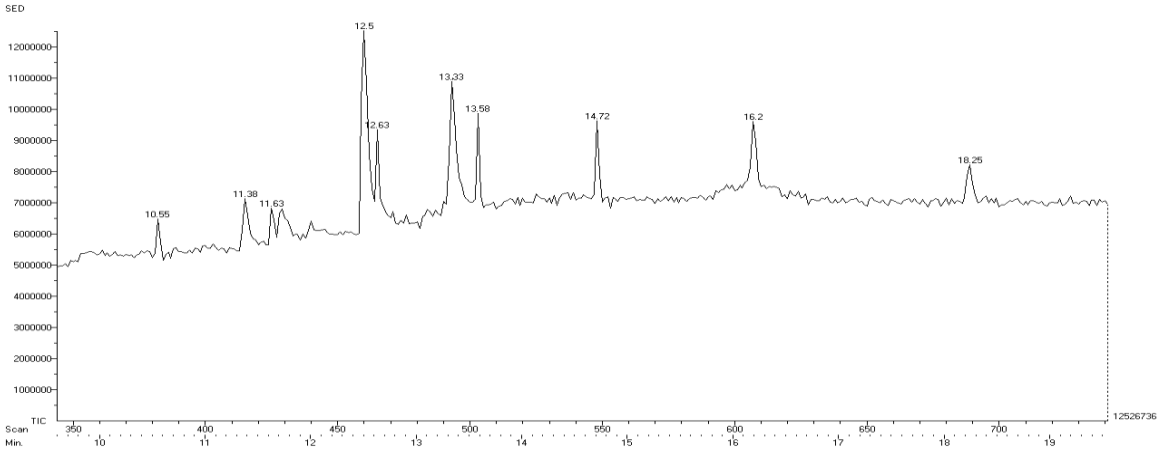


Figure 3: GC-MS profile of prickly custard apple seed.

prickly custard apple peel it was shown in (Table.3 and Graph.2). The comparison of Gallic acid and the quercetin the great difference was noted in the peel and pulp of prickly custard apple. It may give the various antioxidant effects to the fruit quality.

effect of antibacterial activities

The antibacterial activities test was conducted in various concentrations to investigate their relationship. The results of antibacterial activities examination showed that methanol extract fraction was identified maximum antibacterial effect against *Escherichia coli, S. Sureus, Bacillus Sp*, they were denoted by the existence of inhibition zone around the disc it was shown in the Fig.4,5,6 and 7. The effect was no changes in the peel fraction against *Proteus Sp.* Hence the presence of *Klebsiella Sp*. in peel and seed there is no zone effect was observed the results of the average of inhibition zone diameter of methanol extract fractions of soursop peel, pulp and seed can completely be seen on Table. 3. These studies were well documented by the agar double diffusion method.

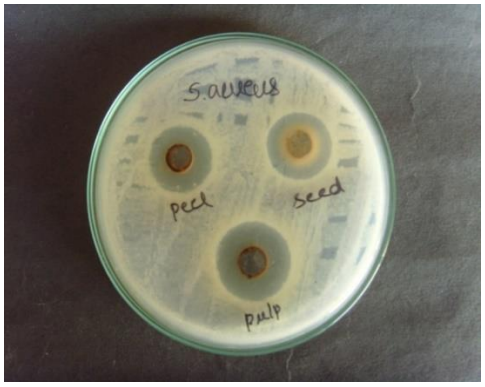


Figure 4: Antimicrobial activity of Annona muricata peel, pulp and seed against *Staphylocucus*

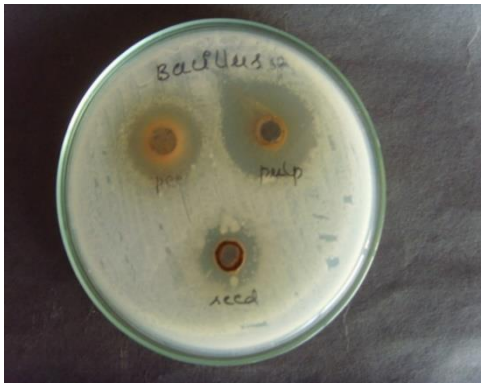


Figure 5: Antimicrobial activity of Annona muricata peel, pulp and seed against bac B*Bacillus*

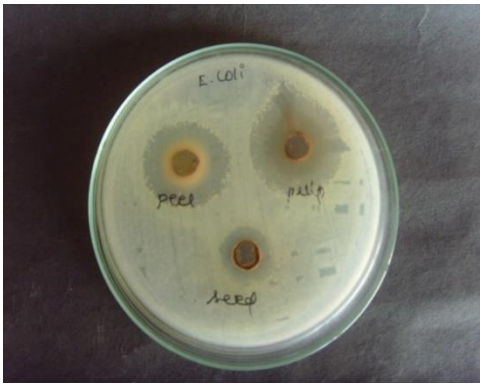


Figure 6: Antimicrobial activity of Annona muricata peel, pulp and seed against *E.coli*

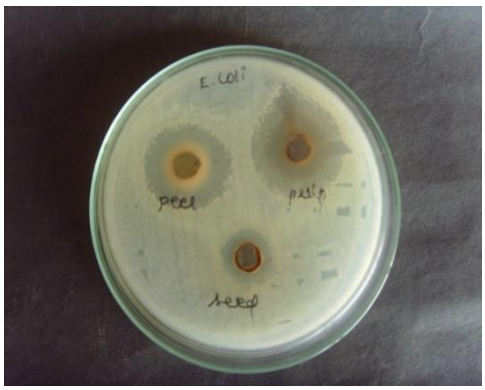
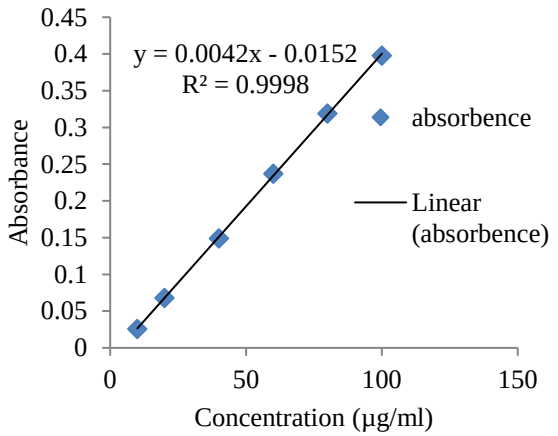
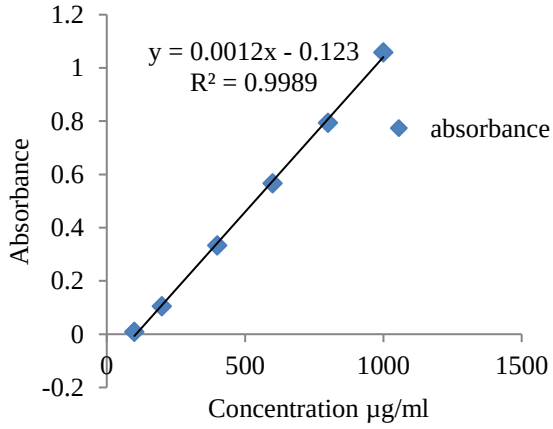


Figure 7: Antimicrobial activity of Annona muricata peel, pulp and seed against *Proteus Sp.*



Graph 1: Absorbance of Gallic acid standard Absorbance: Peel= 0.453, Pulp= 0.437; Content Peel=222.952mg/gm, Pulp= 215.33mg/g



Graph 2: Absorbance of quercetin standard Absorbance: Peel= 0.138, Pulp= 0.104 Content Peel= 261mg/gm, Pulp= 227mg/gm

DISCUSSION

The present investigation is carried out in the fruit of Soursop, the methanolic extract showed antibacterial activity it concluded that the identified phenolic and flavonoids compounds is found to be effective antibacterial activity against *E. coli, S.aureus, Bacillus, proteus Sp. Klebsiella Sp.* Well documented by agar diffusion method. The bioactive compound Dasycarpidan – 1- methanol, acetate (ester) present in both peel and pulp and it was observed and the role was may regulate the fruit development and also it was coordinate with the antimicrobial activity. The present result consistent with previous investigation on bioactive compounds in the Annonaceae family is growing rapidly; acetogenin compounds from Annonaceae type were reported to have toxicity that is effective against insects of several orders

such as Lepidoptera, Coleoptera, Homoptera and Diptera[13]. In addition some of the research group reported that Annonaceae family contains acetogenin that are larvicidal, acetogenin also acts as an insecticide, acaricide, antiparasitic and bactericidal[14,15]. Other variety like *A. muricata* Linn. (Soursop) seed extract contain annonacin, bullatacin, annonin VI, goniothalamin and sylvaticum act as insecticides. [16, 17] Based on the previous reports the present study was done the antimicrobial activity against the microbes. It was revealed that the *Annona muricata* extract have a wide range of activity against a microbes responsible for the most common diseases. These promissory extract open the possibility of finding new clinically effective antibacterial compounds. The present study of peel and pulp of *Annona muricata* forms a primary platform for further phytochemical and pharmacological studies.

CONCLUSION

Prickly custard apple *(Annona muricata)* peel and pulp extracted used by methanol and ethyl acetate. Methanolic extract was given good result compared to ethyl acetate extract. This study identified bio active compounds in specifically Dasycarpidan – 1- methanol, acetate (ester) present in both peel and pulp and it was identified as best antibacterial agent by double diffusion method. The present study analyzed total phenol and flavonoids content in prickly custard apple give better antimicrobial activity of peel and pulp extract against *E. coli, S. Sureus, Bacillus Sp., Proteus Sp., Klebsiella Sp*. The present study to reveal that the microbes' reduction test was confirmed by agar well diffusion method. The present finding supports the peel and pulp of prickly custard apple have effective antimicrobial activity and also the coordination of the bioactive compound may play the important role for the antimicrobial activity. Hence, the presence of the Dasycapidan-1-Methanol, acetate (ester) compound may involve the stimulator for the antioxidant properties. Hence the recommendation of the study needs further confirmation to evaluate the additional importance of the part of the fruit and therapeutic effect of the prickly custard apple and also to determine their full spectrum of efficacy.

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