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Chemoprofiling and Analgesic Effect of Methanol Leaves Extract and Fractions of *Carica papaya*

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ABSTRACT

The present study was carried out to identify the chemical compounds present in the partitioned extracts of *C. papaya* dried leaves using GC-MS technique and also to evaluate the analgesic activity of the methanol crude extract of the dried leaves obtained via hot method in mice using two laboratory models namely: hotplate latency assays and acetic acid induced writhing. The extract given orally (50-200 mg/Kg), increased the reaction time from 2.5 ± 0.6 to 5.3 ± 1.2 seconds in the hot plate model which showed a significant, dose dependent analgesic activity when compared with the control ($p<0.05$ and $p<0.01$). Likewise, the extract at the same doses demonstrated analgesic activity by reducing the writhing numbers from 119.6 ± 26 to 68.8 ± 62. From the GC-MS analysis, the majority of the compounds identified are fatty acids and the major chemical constituents of the four partitioned extracts (methanol, petroleum ether, chloroform and ethyl acetate partitioned extract) were n-Hexadecanoic acid, 9-Octadecenoic acid (E)-, Octadecanoic acid Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester. Other chemical constituents identified were Hexadecane, Tritetracontane, Pentadecane2,6,10,14-tetramethyl, Dodecane, 2,6,10-trimethyl, Octacosane, Palmitoleic acid, 2-methylhexacosane, l-(+)-Ascorbic acid 2,6 dihexadecanoate, Tetrapentacontane, Oleic Acid, Dichloroacetic acid, tridec-2-ynyl ester, erythro-9,10-Dibromopentacosane, Cholestan-7-one, cyclic 1,2 ethanediylacetal, Oleoylchloride, Methyl 9-methyltetradecanoate, 15-Hydroxypentadecanoic acid, Eicosanoic acid, Cyclononasiloxane, octadecamethyl,9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl, 2,3-Dihydroxypropyl elaidate, Pentadecanoic acid, 14-methyl-, methyl ester and Tetradecanoic acid. Many of the compounds identified have antioxidant, hypocholesterolemic, nematicidal, pesticide, antiandrogenic, haemolytic, anti-inflammatory/analgesic, cancer preventive, dermatitigenic, hypotensive, anemiagenic and insectifuge activity which justify the folklore use of the leaves in traditional system to cure various ailments. The results suggest that the methanol leaves extract of *C. papaya* has significant analgesic activity.

Keywords: Analgesic activity, *C. papaya*, GC-MS, Compounds, Mice.

INTRODUTION

Medicinal plants are used in traditional medicine due to their therapeutic potentials and the quest to develop novel medicinal agents from them have been the reason for renewed interest in them among scientist. *Carica papaya* is a power house of many nutrients. The fruits are juicy in taste and enriched with antioxidant nutrients like carotene, vitamin B,

vitamin C, folate, flavonoids, pantothenic acids and minerals such as potassium and magnesium. They are also a good source of fibre, playing an important role to maintain the functions of cardiovascular system and protection against colon cancer [1-5].

C. papaya contains many bioactive components, including Chymopapain and papain which are important proteolytic enzymes found in the milky white latex that is used in the treatment of several digestive disorders. Additionally, the plant also contains terpenoids, eugenol, thymol, saponins and alkaloids. *Papaya* latex is used in the management of dyspepsia and applied externally to burns and scalds. The milky white exudate is antagonistic to fungal growth, especially *Candida albicans*, thus it is used in folk medicine to treat skin eczema caused by this fungus. The leaves are inimical to intestinal worms and have been used successfully for the treatment of boils. Some people use the smoke from dried *papaya* leaves for the purpose of alleviating asthma attacks.

Pain is an unpleasant sensation localized to a part of the body. Pain perception is a normal physiologic response mediated by healthy nervous system (Fields and Martins, 2008). Analgesic agents are used in numerous diseases for alleviating pain. Most analgesic drugs, accessible in the market, exhibit an extensive range of adverse effects including gastrointestinal disorders, kidney problems, and other unwanted effects. This situation highlights the need for the search into safer and more effective analgesic agents [6-8].

To the best of our knowledge, the chemical profiling of this plant have not been previously reported in literatures and there is a dearth of knowledge in the analgesic activities of this plant considering the fact that the same plant from different localities might contain different phytoconstituents.

MATERIALS AND METHOD

Materials

All solvents were of analytical grade. They were obtained from local suppliers and were used without further purification.

Collection and identification of *C. papaya*

The leaf of the plant was collected from University of Benin, Nigeria in April 2016 during rainy season when weed beds were in their maximum densities. The plant was identified by Pharm. Uwumarongie O.H. of the Department of Pharmacognosy, University of Benin where a voucher specimen was deposited and assigned number UBN/PCG/987. They were washed with water, sundried and grounded to fine powder with the aid of a mechanical grinder. The powdered sample was kept in clean closed containers till extraction [9-12].

Extraction and partitioning of the dried powdered sample

The powdered leaf (450.00 g) was extracted with 2.5 litres of methanol using soxhlet apparatus for 72 hours. This was further reduced to dryness on a thermostatically regulated hot water bath at 40^{o}C to yield 26.53% extract [13-16].

The residue was then partitioned into 500 ml of chloroform, petroleum ether and ethyl acetate. These were also reduced to dryness to obtain 42.52%, 46.35%, and 38.54% yield respectively. The crude and partitioned extracts were preserved in refrigerator ($\pm 4^{0}$C) until further analysis.

Analgesic studies

Analgesic effect of papaya was carried out using adult mice of either sex weighing 16-30 g. They were obtained from the animal house, Department of Pharmacology, University of Benin, Benin-City, Nigeria. All animals were allowed to acclimatize for two weeks, exposed to natural lighting conditions and room temperature and allowed access to food and water *ad libitum* Analgesic activity in mice was assessed using chemically and thermally induced pain, using acetic acid induced writhing model (Ghosh 1984) and hot plate assay (Eddy and Leimbach 1953), respectively [17-20].

Analgesic screening using hot plate method

The animals were randomly allotted into five groups each comprising of 5 mice and allowed to acclimatize on the hot place apparatus during which it was off before conducting the experiment.

The groups comprised of: Group A (negative control) were given 5 ml/kg of distilled water orally and then placed on a hot plate maintained at 55^{0}C $\pm$ 1^{0}C one after the other and the time taken for the mouse to respond to the thermal stimulus (Usually by jumping or paw licking) was noted as the latency or the reaction time (in seconds) at an interval of 30 minutes starting

from 0 minute to 120 minute.

Group B (Positive control), were given 10 mg/kg of pentazocin intraperitoneally and then placed on a hot plate maintained at $55^0C \pm 1^0C$ and the time taken for the mouse to respond to the thermal stimulus (Usually by jumping or paw licking) was noted as the latency or the reaction time (in seconds) at an interval of 15 minutes starting from 0 minute to 60 minute [21,22].

Group C, D and E (Test groups), were given 50 mg/kg, 100 mg/kg and 200 mg/kg of the papaya extract orally respectively and then placed on a hot plate maintained at $55^0C \pm 1^0C$ and the time taken for the mouse to respond to the thermal stimulus (Usually by jumping or paw licking) was noted as the latency or the reaction time (in seconds) at an interval of 30 minutes starting from 0 minute to 120 minute.

Analgesic screening using acetic acid induced pain (sigmund method)

The animals were randomly divided into 5 groups of five animals each Control group A, which were given 5 ml/kg distilled water orally and then were injected intraperitoneally by 0.1 mL of 0.6% glacial acetic acid solution in distilled water to induced visceral pain. Animals were placed on metabolic cage and observed for writhing behavior which indicated by stretching of the abdomen. The number of writhing responses was counted every 5 minutes for 30 minutes, starting directly after the acid injection. Group B (Positive control) were given 100 mg/kg of Aspirin orally, after 50 minutes, they were then injected intraperitoneally by 0.1 mL of 0. 6% glacial acetic acid solution in distilled water to induced peripheral pain. Animals were placed under the same condition as the control group. Group C, D and E (Test groups) were given 50 mg/kg, 100 mg/kg and 200 mg/kg of papaya extract orally and treated as above.

Gas chromatography/Mass spectrometry (GC/MS) analysis

GC-MS analysis of the partitioned extracts (chloroform, methanol, ethyl acetate and petroleum ether extract) was carried out on GC-MS- QP2010 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column VF-5MS fused silica capillary column (30.0 m x 0.25 mm x 0.25 µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.5 ml/min and an injection volume of 1 µl was employed (split ratio of 10:1), injector temperature was 240°C; ion-source temperature was 200°C. The oven temperature was programmed from 70°C (isothermal for 3 min), with an increase of 10°C/min, to 240°C, then 5°C/min to 300°C, ending with a 9 min isothermal at 300°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 440 Da. Interpretation of GC-MS spectra was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with spectrum of known component stored in NIST library. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC- MS. The name, molecular weight, retention time and peak area percentage of the test materials was ascertained [23,24].

Statistical analysis

The data were analyzed for statistical significance by one- way analysis of variance (ANOVA) using Graph Pad Instat, version 4.5, software. Values of $p<0.001$ were considered statistically significant.

RESULTS

The result showing the showing the writhing numbers for the treatment groups is presented in Tables 1 and 2.

Table 1: Writhing numbers of treatment groups.

	Number of writhes in 30 min						
Treatment group	**1**	**2**	**3**	**4**	**5**	**∑**	**Mean ± SD**
control group A	133	126	148	112	79	578	115.6 ± 27
Aspirin dose of 100 mg/kg	0	113	63	56	57	289	57.8 ± 40
Group C 50 mg/kg of extract	114	87	125	104	74	504	100.8 ± 41
Group D 100 mg/kg of extract	74	0	124	101	138	437	87.4 ± 55

Group E 200 mg/kg of extract	91	112	135	0	6	344	68.8 ± 62

SD = standard deviation

Table 2: The effects of the methanol leave extract of *C. papaya* on mice in the hot plate test.

Average latency time in seconds						
Treatment group	**0**	**30**	**60**	**90**	**120**	**Mean ± SD**
control group A	3.56	1.9	2.26	2.36	2.48	2.5 ± 0.6
Pentazocin 10 mg/kg	2.84	3.96	4.34	5.98	3.96	4.2 ± 1.1
Group C 50 mg/kg of extract	2.3	2.72	3.54	3.24	5.92	3.5 ± 1.4
Group D 100 mg/kg of extract	2.56	6.7	5.72	4.52	6.16	5.1 ± 1.6*
Group E 200 mg/kg of extract	4.6	6.84	3.76	5.98	5.12	5.3 ±1.2**
SD = standard deviation, *P < 0.05, **P < 0.01						

GC-MS analysis

The result of the GC-MS and the spectra of the fractions are attached as appendix (Figure 1).

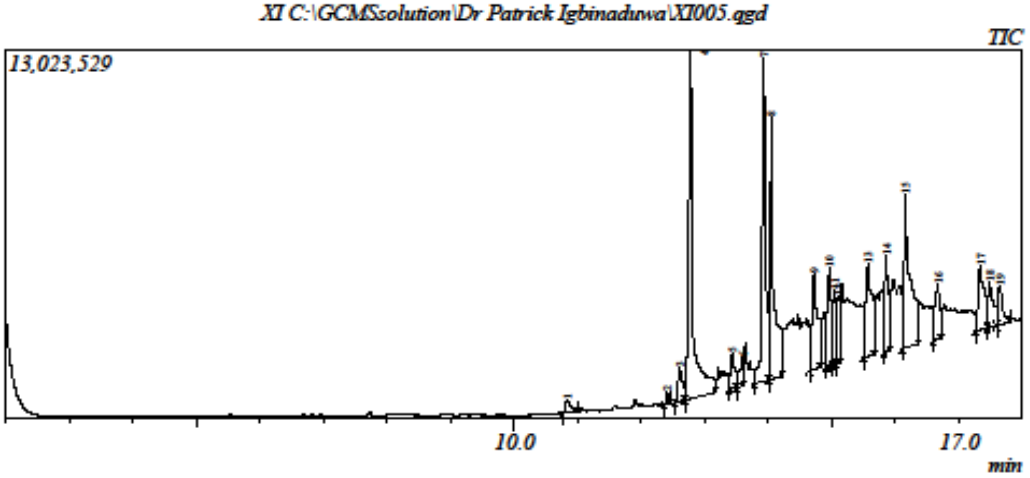
30/07/2016 08:25:23 GCMS-QP2010SE SHIMADZU,JAPAN

SHIMADZU TRAINING CENTRE FOR ANALYTICAL INSTRUMENTS (STC) LAGOS

Sample Information

Analyzed by	: $Ben OS
Analyzed	: 30/07/2016 04:11:24
Sample Type	: Unknown
Level #	: 1
Sample Name: XI	
Sample ID	: XI
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 5
Injection Volume	: 1.00
Data File	: C:\GCMSsolution\Dr Patrick Igbinaduwa\XI005.qgd
Org Data File	: C:\GCMSsolution\Dr Patrick Igbinaduwa\XI005.qgd
Method File	: C:\GCMSsolution\Dr Patrick Igbinaduwa\Dp.qgm
Org Method File	: C:\GCMSsolution\Dr Patrick Igbinaduwa\Dp.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\Ben1 03-05-2016.qgt
Modified by	: Admin
Modified	: 30/07/2016 08:05:01

XI C:\GCMSsolution\Dr Patrick Igbinaduwa\XI005.qgd



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	10.838	10.758	11.017	2811808	0.86	433933	0.63	6.48		Tetradecanoic acid
2	12.410	12.358	12.450	669186	0.20	414947	0.60	1.61	V	Methyl 9-methyltetradecanoate
3	12.609	12.533	12.683	5666118	1.72	1233435	1.80	4.59	V	Palmitoleic acid
4	12.781	12.683	13.175	59023227	17.95	12323336	17.96	4.79	V	n-Hexadecanoic acid
5	13.434	13.383	13.500	5340027	1.62	1265519	1.84	4.22	V	l-(+)-Ascorbic acid 2,6-dihexadecanoate
6	13.601	13.500	13.617	4717854	1.43	1004734	1.46	4.70	V	Dichloroacetic acid, trideo-2-ynyl ester
7	13.933	13.775	14.008	45285144	13.77	11441060	16.68	3.96	V	9-Octadecenoic acid, (E)-
8	14.048	14.008	14.225	38812014	11.80	9285586	13.54	4.18	V	Octadecanoic acid
9	14.717	14.658	14.825	20559795	6.25	3395057	4.95	6.06	V	Hexadecanoic acid, 2-propyl-, methyl ester
10	14.965	14.917	15.008	13847922	4.21	3465317	5.05	4.00	V	15-Hydroxypentadecanoic acid
11	15.041	15.008	15.075	9417790	2.86	2623972	3.82	3.59	V	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-
12	15.100	15.075	15.133	7902656	2.40	2380039	3.47	3.32	V	erythro-9,10-Dibromopentacosane
13	15.573	15.500	15.667	22404776	6.81	3289623	4.80	6.81	V	9-Octadecenamide, (Z)-
14	15.856	15.825	15.917	13860399	4.21	3423198	4.99	4.05	V	Cyclononasiloxane, octadecamethyl-
15	16.159	16.108	16.367	37798134	11.49	5403620	7.88	6.99	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxym
16	16.667	16.600	16.717	9826659	2.99	1965861	2.87	5.00	V	erythro-9,10-Dibromopentacosane
17	17.335	17.267	17.450	14608693	4.44	2252131	3.28	6.49	V	9-Octadecenoic acid (Z)-, 2,3-dihydroxypro
18	17.486	17.450	17.600	9041957	2.75	1601178	2.33	5.65	V	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-
19	17.646	17.600	17.817	7272162	2.21	1401046	2.04	5.19	V	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1
				328866321	100.00	68603592	100.00			

Library

1 / 20

Figure 1: The result of the GC-MS.

DISCUSION

The analgesic potential of *C. papaya* was studied using both peripheral (non-narcotic) and central (narcotic) type of pain models along with GC-MS analysis of the phytochemical constituents of the extract. *C. papaya* pre-treatment markedly reduces the painful response produced by acetic acid, manifested as writhing at the employed doses. Pain is a complex process mediated by many physiological mediators such as prostaglandins, bradykinin, substance P etc. In the acetic acid induced writhing model the constrictions induced by acetic acid in mice results from an acute inflammatory reaction with production of PGE2 and PGF2α in the peritoneal fluid. Therefore, it is likely that *C. papaya* might suppress the formation of these substances or antagonize their action for exerting analgesic activity. The hot-plate test is commonly used to assess narcotic analgesics or other centrally acting drugs and it was selected for use in this study because of its sensitivity to strong analgesics and very limited tissue damage. Furthermore, it utilizes phasic stimulus of high intensities mimicking responses in conditions that involve high threshold pain of short duration and its use to evaluate analgesic drugs on central pain by means of thermal stimulus. The results of the present study show that the methanol leaf extract of *C. papaya* obtained via hot method can significantly inhibit responses to thermal stimuli and acetic acid induced pain. The inhibition in both models was dose dependent (there was an increase in analgesic property of the extract with increase dose) thus, showing that the extract, at the doses administered, showed a significant analgesic activities ($p<0.05$ and $p<0.01$). This is in line with the study carried out by Owoyele et al., but also shows that the analgesic activity of methanol extract is dose dependent.

In GC/MS analysis, the four partitioned extract showed several peaks from the chromatogram indicating several compounds. The methanol extract showed thirteen peaks, petroleum extract showed twenty two peaks, the chloroform extract showed sixteen peaks and the ethyl acetate extract showed 21 peaks. Showing that, the petroleum extract gave the highest number of compounds. The compounds found in the four partitioned extract were majorly fatty acids. The major chemical constituents of the four partitioned extracts (chloroform, methanol, ethyl acetate and petroleum ether partitioned extract) were n-Hexadecanoic acid (22.68%, 19.03%, 19.55% and 18.50% peak area respectively), 9-Octadecenoic acid (E)- (17.07%, 15.56%, 14.91% and 15.11% peak area respectively. Octadecanoic acid (13.17%,14.17%, 10.23% and 7.93% peak area respectively), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (12.92%,12.46%, 13.23% and 11.54% peak area respectively) which have anti-inflammatory/analgesic properties. Other chemical constituents identified were Hexadecane, Tritetracontane, Pentadecane2,6,10,14-tetramethyl, Dodecane, 2,6,10-trimethyl, Octacosane, Palmitoleic acid, 2-methylhexacosane, l-(+)-Ascorbic acid 2,6 dihexadecanoate, Tetrapentacontane, Oleic Acid, Dichloroacetic acid, tridec-2 -ynyl ester, erythro-9,10-Dibromopentacosane, Cholestan-7-one, cyclic 1,2 ethanediyl acetal, Oleoyl chloride, Methyl 9-methyltetradecanoate, 15-Hydroxypentadecanoic acid, Eicosanoic acid, Cyclononasiloxae, octadecamethy l,9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl, 2,3-Dihydroxypropyl elaidate, Pentadecanoic acid, 14-methyl-, methyl ester and Tetradecanoic acid.

CONCLUSION

In conclusion, the methanol leaf extract of *C. papaya* obtained via hot method at doses of 50 mg/kg, 100 mg/kg and 200 mg/kg offered a protection against thermal stimuli (central pain) produced by the hot plate method and acetic acid induced peripheral pain. Therefore, there was a significant analgesic activity ($p<0.05$, $P<0.01$). The presence of various bioactive compounds justifies the use of the leaves of *C. papaya* for various ailments by traditional practitioners and also, most of the chemical compounds responsible for bioactivities are not thermolabile.

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