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Evaluation of Cochorus Olitorious Derived Polymer as an Excipient in Micro/Nano Suspension Formulation of Metronidazole

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ABSTRACT

Crude C.olitorious gum was extracted by the Soxhlet extraction method then subjected to microbial, elemental and proximate analysis. From the microbial analysis, the amount of microorganism obtained after 24 hours culture in a plate count agar was 0.25 x 10^3 .This level of growth could be related to non-sterility and the environmental conditions under which the raw material was processed, of which in some cases the leaves were air dried. Elemental composition of the gum includes magnesium (5402mg/kg), calcium (5267mg/kg), iron (1435mg/kg), sodium (2145mg/kg, zinc (120mg/kg) and lead (nil). This result indicates that the gum is free from deleterious materials such as lead and other similar materials. The acute toxicity test on the C. olitorious gum using animal species indicated no death after two weeks of observation showing that the gum and materials used for extraction are compatible with the animal physiological system. Purification by centrifugation of the crude C.olitorious gum was carried out after preliminary analysis and the purified gum expunged from the supernatant layer using organic solvent (acetone). The percentage yield of the purified gum from the crude gum was 20.0%w/w depicting the presence of about 80% of other chemical and extraneous materials in the crude C. olitorious gum.

Key Words: C.olitorious gum, *Corchorus Olitoriou.*

1. INTRODUCTION

Nature has provided mankind with varieties of materials to help improve their health either directly or indirectly. Gums and mucilage's especially from the natural sources have in recent times been widely used for conventional and novel dosage/ drug administration. They have advantages over synthetic ones because of their chemical inertness, non -toxic, less expensive and wide availability and applicability [1]. Natural polymers can be modified in different ways to obtain tailor made materials for drug delivery systems and thus can compete with available synthetic excipients. The type of compounds contained by the polymers influences the properties of different gums. Increased straight chain polysaccharides are known to occupy more spaces and are observed to be more viscous than highly branched compounds of same molecular weight while the branched compounds form stable gels more readily and are more stable because extensive interaction along the molecular chain is impossible [2].

Increased interest in the development of new pharmaceutical excipients such as in compatibility consideration and the need to develop better excipients that will enhance faster and stable product formulation has necessitated the approach for new drug product delivery system and for the administration of biotechnology using new excipients that will further avoid the inconvenience of multiple daily dosing and improvement in therapeutic action of certain drugs which probably may occur by possible conjugation with water soluble bio-compatible excipients [3]..

Natural gums could also be modified to overcome certain draw backs like uncontrollable rate of hydration, thickening, drop in viscosity on storage and microbial contamination [4].

Cochorus olitorious plant is an Afro- Arabian plant with genus Cochorus and family Tilaceae. The plant is an annually branched herb 90 – 120 cm tall pan- tropical in distribution often a weed than a cultivar. It could tolerate an annual temperature of 16.8 to 27±5^{o}c, rainfall of 4.0 to 42.0 dm^{3} and pH of 4.5 to 8.2 and usually harvested in between September to early November [5]. The *Cochorus olitorious* plant is commonly known as *ewedu* and *ahihiara* respectively among the Yoruba and the Ibo tribes of Nigeria. The leaves of the plant are usually made into a mucilaginous (somewhat slimy soup in some West African cooking tradition [6]. The extraction process of the crude gum, physico chemical characterization and investigation into the suspending properties were carried out as in part 1 and 2 of this series [7].

Pharmaceutical micro/nano suspension formulations are preparations containing finely dispersed colloid bi-phasic solid drug particles in aqueous vehicle size equal to or below 1µm without any matrix material [8], stabilized by surfactants and polymers [9] [10]. The nano/micro suspension could be prepared by suitable methods for drug delivery and application through various routes (oral, topical, parenteral, pulmonary etc. [11] [12], improving drug safety and efficacy.

The aim of this study is to process and evaluate the crude gum obtained from the dried leaves of *Cochorus olitorious* plant as an excipient in micro/nano suspension drug delivery system.

2. EXPERIMENTALS DETAILS

Materials: Materials used are all of Pharmaceutical grades and includes: acetone (Sigma Aldrich, Germany) Tragacanth, NaCMC, crude gum extract form *C.olitorious* (University of Port Harcourt, Nigeria. Pharm technology laboratory), albino rats of wistar strain, pH meter (Hanna H 9610 USA), Table centrifuge (Pec medical USA), , Electronic digital weighing balance (Adventurer TM AR 21030 China).

2.1 METHODS

2.1 **Elemental analysis of crude C.olitorious gum**

A 0.1g of extracted C.olitorious gum sample was burnt to ash in a muffle and subjected to a temperature of 630°c for 3hours. The ash sample was dissolved in 10ml Conc. HCl and heated in an electro-thermal heater/ hot plate. The solution of the ash was diluted in 50ml distilled water.

The solution was analyzed for metal ion by atomic absorption spectrophotometer at the following wavelength, Lead (Pb) 283.3nm, magnesium (Mg) 285.2nm, iron (Fe) 288nm, zinc (Zn) 213.8nm, Sodium (Na) 589nm and calcium (Ca) 422.7nm.

2.2 Proximate analysis of crude *C.olitorious* gum

The crude *C.olitorious* gum was subjected to analysis for presence of: carbohydrate (CHO), moisture contents, lipids, total ash, acid insoluble ash and protein contents adopting the AOAC method of analysis.

2.3 Microbial load determination

The powdered crude *C.olitorious* gum was solubilized in distilled water adopting serial dilution technique (1 in 10³ml).

A 1.0ml of the solution was inoculated by streaking method on Mackonkay agar plate then incubated for 24hours at 57°c for further observation.

2.4 Toxicological studies

The crude gum extract was used for acute oral toxicity test studies using albino rats (either sex) of Wister strain to evaluate its toxicity and median lethal dose (L D 50) for 14 days.

2.5 Purification of the crude *Cochorus olitorious* gum

A weighed quantity of crude gum was dispersed in freshly prepared distilled water in the ratio 1:10 of gum to water respectively and allowed to stand for 6 hours.

The mucilage was centrifuged (Pec medical USA) at 3000rpm for 25minutes. The supernatant was expunged and centrifugation of the supernatant repeated thrice then resultant product treated with acetone to expunge the entrapped gum. The gum extract was washed severally with acetone to remove excess moisture and impurity. The purified gum was dried in an oven (Memmert u-27,

England) at 40-50°c to constant weight, pulverized and passed through an 180µm sieve aperture, weighed and stored in a desiccator for further use.

2.5.1 Determination of percentage yield of purified *C.olitorious* gum

The weight of the crude gum extract was noted and after centrifugation, the final weight of the gum entrapped in the supernatant after washing and drying was noted.

$$\text{Percentage yield} = \frac{\text{weight of purified of purified gum}}{\text{Initial weight of crude gum}} \times 100 \quad \text{--------- (1)}$$

2.5.2 pH Determination of *C.olitorious*

A 0.1g of purified *C.olitorious* gum powder was dispersed in 10, 20 and 50ml of distilled water with agitation.

The mixtures were tested for pH using pH meter (Hama 96107, USA) in triplicate and the mean pH recorded.

2.5.3 Purity assessment of *C.olitorious* gum

Sample of the purified gum was weighed to the nearest 0.1mg and analyzed for purity (determination of the melting point) by use of differential scanning calorimetry (DSc) (Seiko 5sc/5200, England).

2.5.4 Compatibility studies of purified C.olitorious gum

This was carried out by the use of Fourier transform infrared analysis method (FTIR) to verify the possibility of interaction between active pharmaceutical ingredient API such as metronidazole and the excipients. A drop of the liquid sample was placed on the surface of a well-polished cell plate containing KBr. This was also placed in a second plate which was made to lie on the first plate so as to spread the liquid in a thin film or layer between the plates and the plates clamped together.
The liquid on the edge of the plate was cleaned and the sandwiched plate mounted on the sample holder in a path of the IR beam and the spectrum was run. After the analysis the plates were cleaned with isopropanol and the returned back to the desiccator.

1.5.5 Solubility profile of purified C.olitorious gum in various solvent:

A 0.1g of the purified C.olitorious gum was dispersed in 10, 20 and 50ml of various solvents and homogenized at 3500rpm for 10, 20, 30, 40, 50 and 60 minutes and extent of solubility determined after one hour of settlement.

Statistical evaluation: All data obtained were analyzed statistically for the mean, standard deviation and analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

1.1 Determination of elemental composition of *C.olitorious* gum

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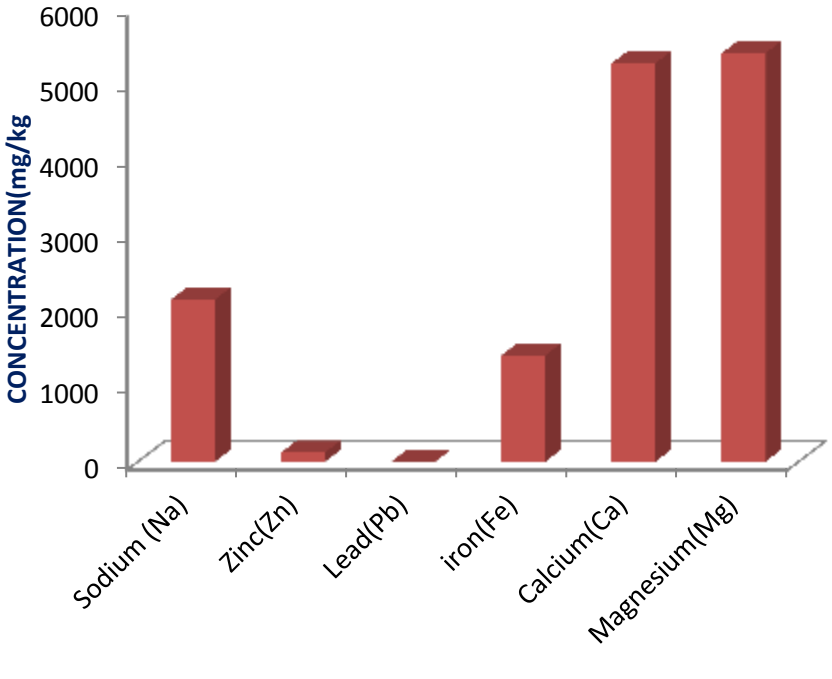

FIG. 1: ELEMENTAL COMPOSITION OF *C.olitorious* GUM

1.2 Proximate analysis of crude *C.olitorious* gum

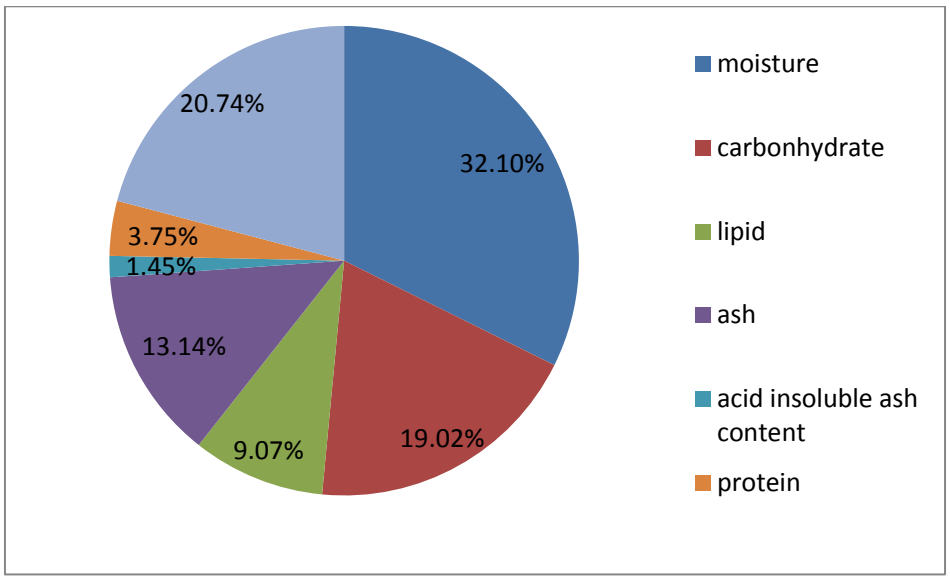

Fig 2: Pie chart of Proximate Analysis and Percentage chemical composition of crude *C.olitorious* gum

MICROBIAL LOAD AND GROWTH COUNT

Result of the microbial load test: Growth count of microorganism was **0.25 X 10^3**/ml.

TABLE 8: Acute Toxicity Test of crude *C. olitorious* gum.

Group Number	Mean weight of rat before mucilage of gum administration (g)	Conc. of gum extract administered (mg/(ml)	Mean Weight of rats (g)	Observation after 14 days
A	104	-	104	No death
B	104	0.4g in 1ml	105	No death
C	105	0.8g in 2ml	116	No death
D	103	1.6g in 4ml	105	No death
E	118	3.2g in 8ml	120	No death
F	113	6.4g in 16ml	115	No death

PERCENTAGE YIELD OF PURIFIED GUM

The Percentage yield of purified gum obtained from crude *C.olitorious* Gum Extract is **20.0%w/w**

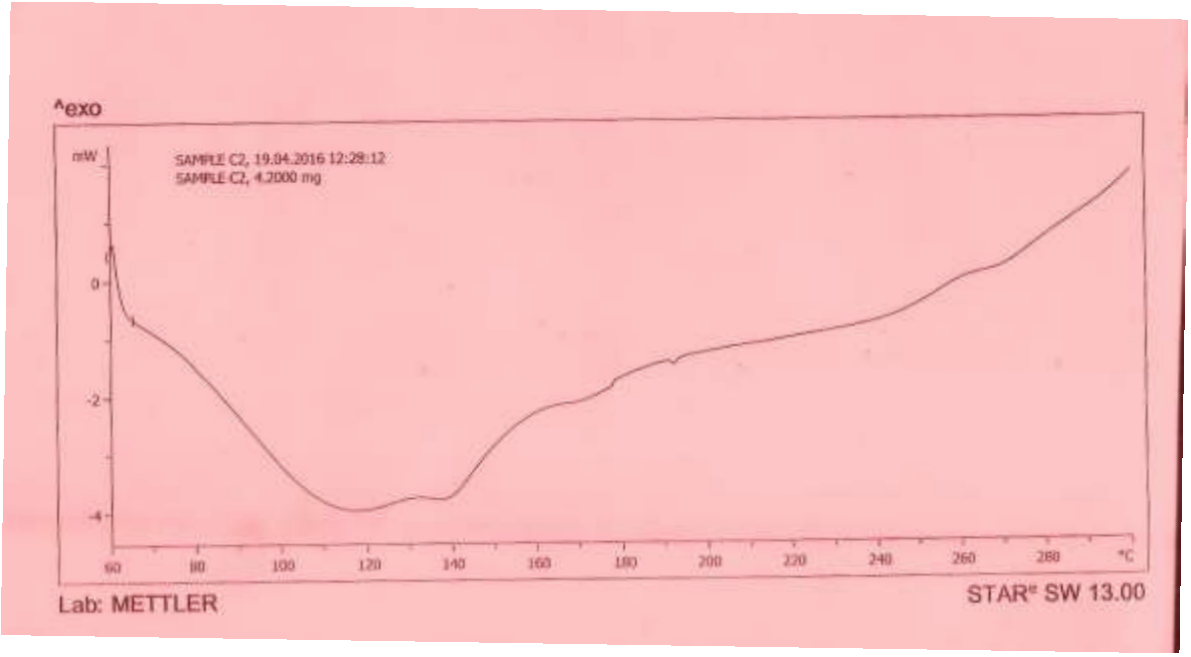

Fig 3: Melting point and Purity Determination of Extracted *C.olitorious* Gum

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TABLE 2: FUNCTIONAL GROUPS AND ABSORBANCE PEAKS IDENTIFIED IN *C. olitorious* GUM

Functional groups	Peak range (cm^{-1})	Peak value (cm^{-1})
O – H	3200 - 3600	3410
C – H stretch	2850 - 3000	2862, 2924
C – H aromatic	1400 - 1600	1411
C = C stretch Amide C = O	1620 – 1680 1640 - 1690	1643 1643
= C - H	670 - 1000	671.25
Ether C - O	1070 - 1150	1149.61
C –N (Nitrile)	2210 -2360	2330
Ester C – O stretch	1000 – 1300 2 bands or more strong	1149.61, 1242.2

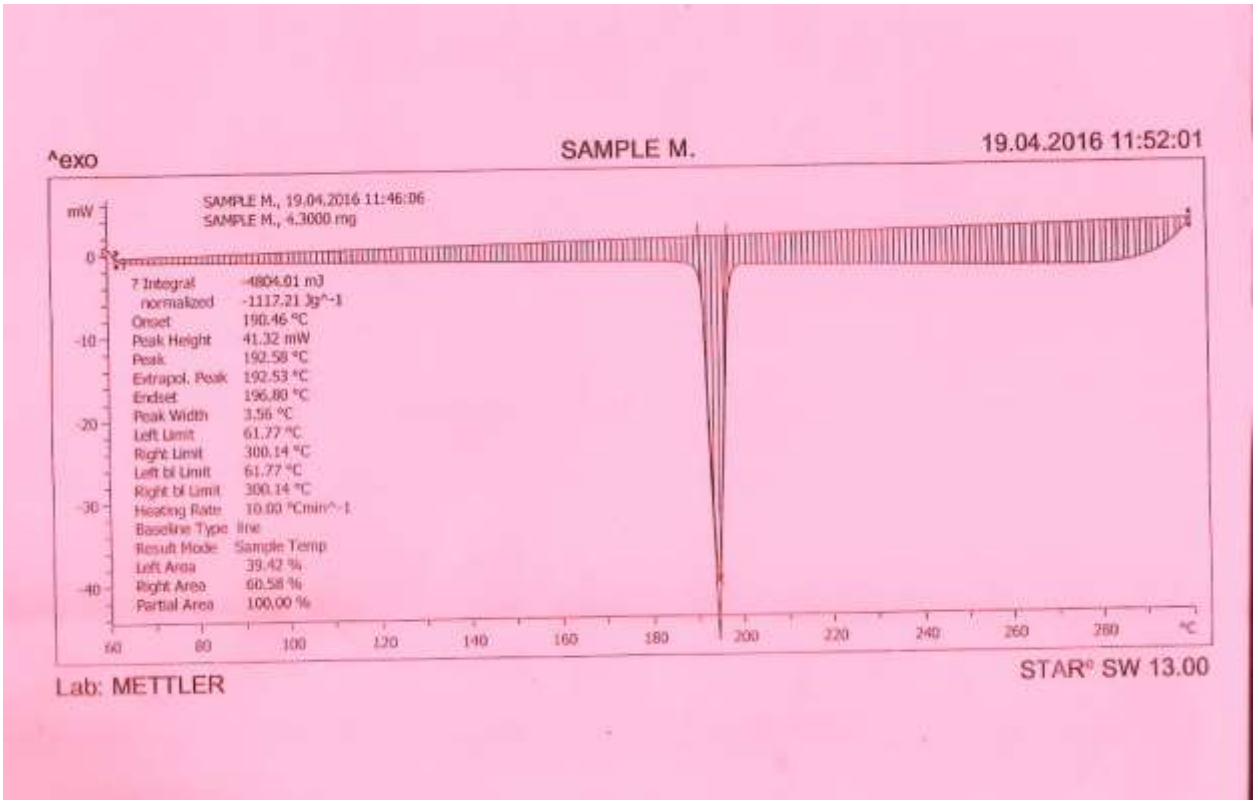
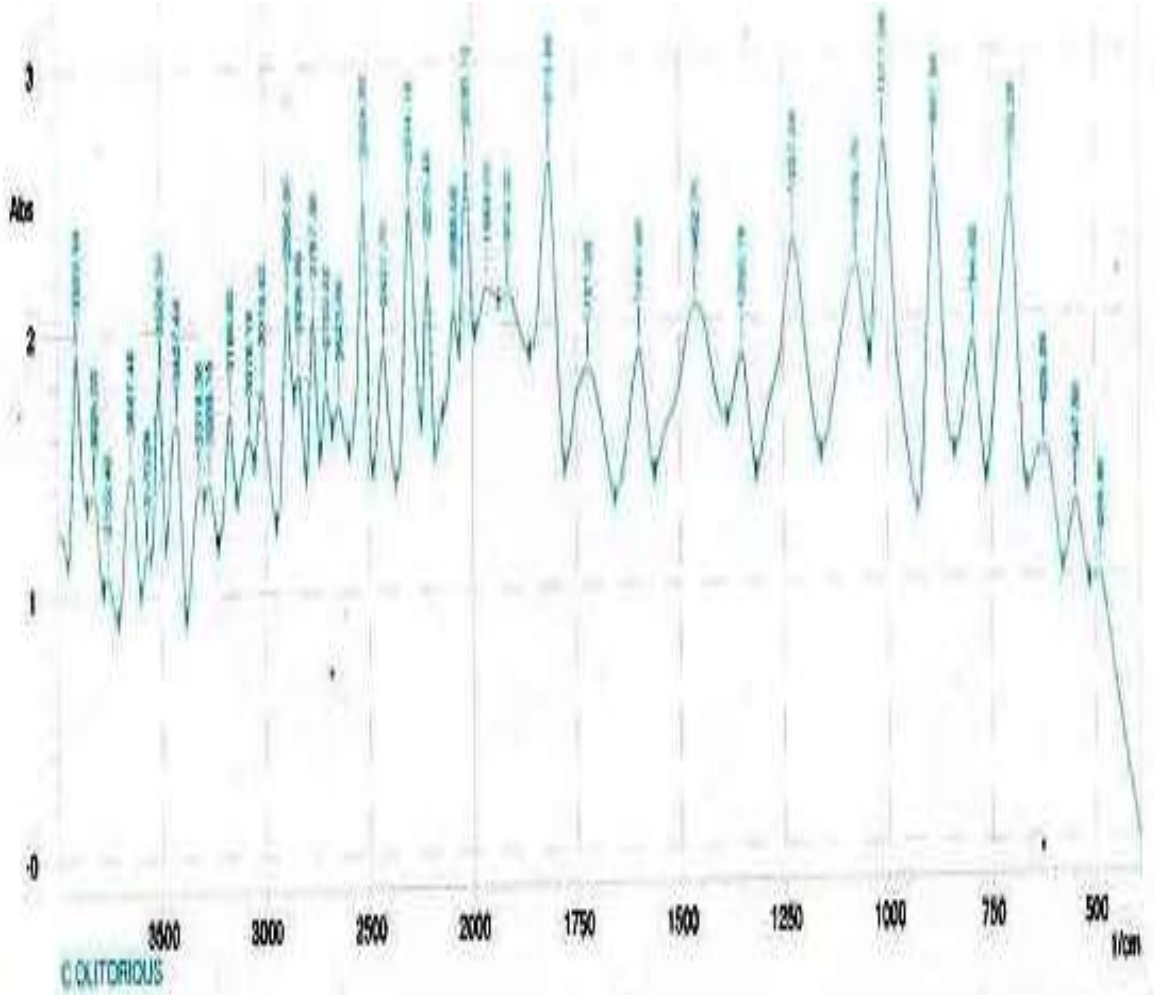


Fig. 4: MELTING POINT AND PURITY STUDY OF MTZ POWDER (METRONIDAZOLE BENZOATE)



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	488.98	1.0001	0.2136	513.14	393.48	70.1911	13.7208
2	547.88	1.2672	0.2725	574.9	517	66.8736	8.3572
3	625.08	1.4824	0.2813	663.68	578.76	114.7783	14.5938
4	702.28	2.4448	1.0802	780.18	687.54	178.1992	51.3291
5	794.82	1.9012	0.4787	837.28	764.04	121.295	18.5458
6	887.56	2.5579	1.1849	926.16	841.24	161.5258	43.7069
7	1011.08	2.8711	0.9766	1041.98	930.02	219.2212	45.1538

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8	1076.7	2.1945	0.4333	1161.82	1045.82	219.5746	24.9352
9	1227.24	2.3034	0.8383	1316.02	1165.48	277.703	58.0632
10	1350.76	1.8741	0.3517	1385.5	1319.88	111.7458	11.8444
11	1462.7	2.0585	0.5123	1559.2	1389.36	303.1511	42.3726
12	1597.8	1.9017	0.5075	1655.7	1563.06	150.2604	22.0214
13	1721.32	1.8327	0.4285	1775.36	1659.56	191.1753	29.2845
14	1813.96	2.6205	0.9743	1860.28	1779.22	171.7879	36.6648
15	1914.32	2.1112	0.0737	1933.62	1864.14	141.0116	2.8875
16	1964.5	2.1467	0.1097	1991.52	1937.48	113.36	3.061
17	2030.12	2.5897	0.6432	2061	1995.38	149.8481	21.9227
18	2088.02	2.0584	0.2123	2142.06	2064.86	145.2276	6.3431
19	2215.4	2.1947	0.6167	2246.28	2180.66	123.8197	20.7243
20	2304.18	2.4561	0.9154	2365.94	2250.14	225.598	47.6349
21	2427.7	1.9231	0.481	2477.88	2369.8	179.882	24.2859
22	2524.2	2.4844	0.9928	2593.68	2481.74	211.4806	43.5718
23	2643.86	1.7125	0.1237	2674.74	2597.54	126.3604	4.4611
24	2705.62	1.7666	0.184	2732.84	2678.6	90.9784	5.2901
25	2767.38	2.043	0.5565	2798.26	2736.5	109.9955	18.0501
26	2836.86	1.8234	0.1848	2856.16	2802.12	90.9802	5.533
27	2890.9	2.1042	0.5315	2944.94	2860.02	147.1155	18.6103
28	3014.42	1.7789	0.355	3049.16	2948.8	156.0487	17.2498
29	3076.18	1.5994	0.132	3134.08	3053.02	122.3596	5.6432
30	3168.82	1.6843	0.3773	3226.72	3137.94	128.8514	14.7855
31	3269.18	1.4167	0.1121	3292.34	3230.58	82.8035	3.8936
32	3319.36	1.41	0.1626	3381.12	3296.2	105.6459	7.6491
33	3427.44	1.6527	0.5953	3473.76	3384.98	124.7745	30.8977
34	3508.5	1.832	0.6592	3550.96	3477.62	109.0733	23.1983
35	3570.26	1.1657	0.0657	3597.28	3554.82	47.5306	1.356
36	3647.46	1.4648	0.495	3709.22	3601.14	133.0721	28.8004
37	3759.4	1.0719	0.0714	3776.7	3713.08	65.9734	2.1957
38	3825.02	1.3963	0.1812	3855.9	3782.56	93.4423	6.806
39	3909.94	1.9302	0.7059	3948.54	3859.76	137.9869	23.5725

Fig.5: FTIR analysis of purified *C.olitorious*

3.1 DISCUSSION

The solubility test for the extracted gum reveals the gum as being insoluble in organic solvent but dispersible in water hence its potential and stability in liquid formulations can only be ensured if aqueous or water miscible solvents are used therefore use of the gum should be avoided or closely monitored in formulations compounded with water immiscible organic solvents like acetone, ethanol, chloroform etc., to avoid precipitation, instability and non- elegance of the drug formulations made with the gum.

Phytochemical confirmatory test of the *C.olitorious* mucilage reveals absence of starch but presence of peculiar carbon hydrate and polysaccharide in the gum.

The proximate analysis of the gum extract from *C.olitorious* plant shows the presence of moisture (32.10%), carbon hydrate (19.02%), fibers (20.75%), ash (13.14%), protein (3.75%), fat (9.07%), and acid insoluble ash content (1.45%). The presence of these proximate principles helps in energy supply and storage, provision of adequate amount of amino acid needed for body synthesis and also assisting to normalize intestinal transit time in humans.

In the physicochemical characterization, the pH of the crude *C. olitorious* gum in water is 6.8 – 7.2. This pH value is within the neutral pH range suggesting that the gum could be a compatible excipient in the formulation of either basic or acidic drugs. This has also been proved by the absence of colour and other physical changes, when the powdered gum was mixed with powders of some active pharmaceutical drugs like - paracetamol, aspirin (acetyl salicylic acid), metronidazole and ascorbic acid and observed for possible physical changes after 12 months.

The amount of microorganism obtained after 24 hours culture of the extracted crude *C.olitorious* gum in a plate count agar was 0.25×10^3. This level of growth could be as a result of the non-sterility and the impact on the environmental under which the raw material was processed where in some cases the leaves were air dried and this could be a good source of contamination although the growth was also believed to been minimal because of the co – processing of the gum with organic solvents such as n-hexane, ethanol and acetone.
Elemental composition of the crude *C.olitorious* gum includes: magnesium (5402mg/kg), calcium (5267mg/kg), iron (1435mg/kg), sodium (2145mg/kg, zinc (120mg/kg) and lead (nil). This result indicates that the gum is free from deleterious materials such as lead and other of such similar materials. This result also indicates that the plant from which the gum was obtained is a good source of nutrient for bone formation and increased body activity such as red blood cell formation with increased iron absorption especially regarding the high magnesium content which basically aids in red blood cell enrichment and enhancement of nutrient supply to the appropriate body sites [13].

The acute toxicity test of the *C. olitorious* gum on rat (wistar strain) indicated no death after two weeks of oral administration and study indicating that the gum and materials used in extraction process are not harmful and did not cause morbidity but show the gum to be rich in nutrients and compatible with the animal physiological system. This compatibility is also being demonstrated by the continuous usage of the *C.olitorious* leaves as a soup thickener in Nigeria especially among the Yoruba tribes, where the sauce is known as *ewedu* soup recognized for its mucilaginous and slimy properties.

Developing a poorly water soluble drug has always been a challenging problem in pharmaceutical drug formulations and to overcome this, the idea of the formulation into nano/micro suspension drug delivery system by incorporation of suitable surfactants and polymer has to be generated. This method aids in formulating the components into a homogenous mixture in an aqueous medium and without allowing for their sedimentation or separation over a long period of time. This formulation technique helps to reduce the frequency of drug administration, enhance effective drug delivery and influence a better therapeutic performance of the active drug component.

For the purpose of this study involving the nano/micro suspension formulation, metronidazole was the chosen as the API because of the following properties: sparing solubility in water (0.1g in 20ml of water), occurrence as high dose drug formulation (200mg and 400mg), association with high partition coefficient (high log p value) and ease of sedimentation when formulated using conventional method of suspension formulation. These properties being critical for nano/micro suspension formulation therefore qualifies metronidazole as a candidate for consideration in the development of such oral drug delivery system.

For the purpose of this drug delivery system to be achieved, an organic or non-aqueous phase is involved and this phase must contain a medium (organic solvent or oil) phase, which must possess such properties as: ability to solubilize the API, partial miscibility with the aqueous medium and must not precipitate the polymer (*C.olitorious* gum) used as the stabilizer. These qualities necessitate the choice of propylene glycol as the organic phase for this study.

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Purification of the crude *C.olitorious* gum was carried out by centrifugation method with the purified gum expunged from the supernatant using organic solvent (acetone). The reason for choosing the supernatant layer is to obtain the pure gum component of the crude gum extract which when dried and powdered will be free from gritty feel, produce particles of good texture that will be nearly equal in size, shape and amorphous in nature.

The FTIR analysis result showed no loss of peak absorbance values of the functional groups such as: O-H, C – H, C = C , C = N, C – C, N – O and 5 – membered ring structure as possessed in the metronidazole structure when powder mixture of the mtz and the purified gum (*C.olitorious*) was analyzed hence the extracted gum is compatible with the API.

4. CONCLUSION

C.olitorious dried leaves consists of a polymeric material insoluble in non- aqueous solvents but soluble in water. Elemental composition of the extracted *C.olitorious* gum reveals presence of essential metal ions responsible for healthy body function but free from deleterious materials hence could be a good source of nutrient.

The method adopted for the purification and the recovery of the purified gum was well chosen and applied. To achieve the formation and stable micro/nano suspension formulations therefore, pH determination, compatibility studies between the API and other excipients along with the particle size reduction of the polymer involving recovery from the supernatant layer should be adopted as these could lead to formulation of a good and homogenous product.

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