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J. Appl. Sci. Environ. Manage. *Dec.*, 2015
Vol. 19 (4) 795 - 801

The Chemical Constituents and Bioactivity of the seed (Fruit) extracts of *Buchholzia Coriacea* Engler (Capparaceae).

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ABSTRACT: *Buchholzia coriacea* Engler (*Capparaceae)* is important in traditional medicine because many parts of the plant have been reported to possess therapeutic potentials. The seeds of the fruits of *Buchholzia coriacea* were extracted with petroleum ether, chloroform and methanol respectively, and the crude extracts were analyzed using GC-MS. The result indicated presence of cyclooctasulphur, oleic acid, (Z,Z)-9,12-octadecadienoic acid, n-hexadecanoic acid, methyl ester (Z,Z)-9,12-octadecadienoic acid, 5-hydromethylfurfural, (E)-9-octadecanoic acid, 1-methyl-pyriolidine-2-carboxylic acid, undecane, palmitoleic acid, stigmasterol, beta-sitosterol and methyl ester 3,5-dicyclohexyl-4-hydroxy-benzoic acid amongst other constituents. Bioactive test of the crude extracts and fractions from column chromatography showed great potential as antifungal agent than antibacterial agent, when tested against isolated fungi and Gram-positive and Gram-negative bacteria. © JASEM

http://dx.doi.org/10.4314/jasem.v19i4.30

KEYWORDS: *Buchholzia coriacea*, Capparaceae, antifungal activity, bioactivity, chemical constituents, fruits

INTRODUCTION

In traditional medicine, the efficacy of herbs is tested and proven and their application has been of great assistance in treatment and prevention of diseases as well as development of new plant derived drugs. Buchholzia *coriacea,* a perennial plant, which belongs to the family *Capparaceae,* grows as a forest tree and it is one of the plants used in herbal medicine due to its medicinal potential. The crushed seeds are used on skin eruption. The bark decoction is also used to wash persons with small-pox (Burkill, 1985). The young leaves are used as a poultice for boils and the seeds used as condiments or as cough medicine (Dalziel, 1937). Previous studies on the plant reported isolation of cyclooctasulphur which had significant antifungal activity on A*spergillus fumigates, Mucor mucedo* and P*enicillium nigricans* (Ojinnaka et al., 1992). The leaves had anthelmintic effects on *Fasciola hepatics* (Ajaiyeoba et al., 2001). The ethanolic extract of *Buchholzia coriacea* was also shown to have antitrypanosomial activity in mice experimentally infected with *Trypanosoma brucei* (Nweze et al., 2009). Mbata et al., (2009) reported that the methanolic extract of *Buchholzia coriacea* seed exhibited good antibacterial activity on some gastrointestinal bacteria; *Bacillus cereus, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Vibrio cholera.* The aim of present work is to detect the chemical constituents of crude extracts of *Buchholzia coriacea* using GC-MS *a*nd to determine the bioactivity of the constituents. To the best of our knowledge, present study reports, for the first time, detection of bioactive constituents from the fruit of *Buchholzia coriacea.*

MATERIALS AND METHOD

*General***:** Evaporation was carried out with Rotavapor R110, (Buchi, Laboratoriums Technik AG CH-9230 FLAWIL/SCHWEIZ). The Gas Chromatography-Mass Spectroscopy (GC-MS) analyses were performed on a Gas Chromatography (GC) ailment 7890A with Mass Spectrometer system 5975. Capillary column HP-5 was used with column length 30m and diameter 0.32. The oven temperature was programmed from 60- 240°C and helium was used as the carrier gas at 1ml/min. The components of different extracts were identified based on the names of the compounds, retention time, area percentage, peak numbers and mass spectra obtained using the data base library of National Institute of Standard and Technology (NIST) Integrator- Chemstation. CC was on a column (length 60cm, diameter 2.5cm) packed with silica gel (60 - 120 mesh).

Plant material. The fruits of *Buchholzia coriacea* were collected from Ojinnaka's compound in Akpulu, Ideato-North LGA, Imo State, Nigeria and were authenticated by Dr. Edwin Wosu, a staff of University of Port Harcourt Herbarium.

Extraction and identification of bioactive constituents. The seeds were deseeded from the fruits, air- dried, at room temperature, for three (3) weeks, then cut into small pieces and milled into fine powder using hand mill. Extraction was done by cold maceration using different solvents (petroleum ether

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60-80^{0}C, chloroform, 60-62^{0}C and methanol, 64.6^{0}C) successively. The milled seeds (1.65kg) were soaked with 2.6L petroleum ether for 72hrs in a 5L aspirator bottle with intermittent shaking, and later soaked again with 1.0L of petroleum ether for complete extraction. This process was repeated using chloroform (2.5L) and methanol (2.5L). The filtrate of different solvents were concentrated using rotary evaporator at 40^{0}C. The petroleum ether crude extract was filtered and the precipitated solid washed with cold petroleum ether to obtain a light- yellow solid crystal of cyclooctasulphur (2.165g) and oil (15.276g). The Chloroform and Methanol extracts gave viscous oil 3.477g and 151.075g respectively. The petroleum ether, chloroform and methanol extracts were screened, using gas chromatography-mass spectrometry (GC-MS) technique to obtain the constituents in Table 1, Table 2 and Table 3 respectively.

Chromatographic analysis. The crude methanol extract (3g), which was eluted with a mixture of petroleum ether in chloroform and then methanol, yielded fractions A (0.139g), B (0.038g), C (0.433g), D (0.495g), E (1.160g), and F (0.0465g) after concentration under reduced pressure (45°C). The antimicrobial activity of fractions A, C, D and E were tested against the microorganisms of interest, together with petroleum ether, chloroform and methanol crude extracts (Tables 4-9).

Isolation and identification of bacteria. The bacteria used for the bioassay test were clinical isolates collected from the laboratory of the Medical Microbiology Department of Braithwaite Hospital, Port Harcourt, Rivers state, Nigeria. The isolates were cultured on both blood agar and chocolate agar and thereafter further purified by subculturing each isolates on a fresh plate of Nutrient Agar (NA) and incubated at 37°C for 24 hours. The isolates were characterized and identified based on the results of gram stain and other biochemical test as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis* and *Pseudomonas aeuroginos* with reference to the Bergey's Manual of Determinative Bacteriology, (1994).

Isolation and identification of dermatophytes. This was done using modified method by Grodmadzki *et al*., 2003. Fungi used were isolated from scrapings obtained from affected feet of a student from the University of Port Harcourt, Rivers state, Nigeria by standard method. Samples were prepared using standard 10fold serial dilution method in buffered saline. Prepared samples were placed on Sabouraud Dextrose Agar plates containing cyclohexamide 4mg/ml, penicillin 20μ/ml, streptomycin 40μ/ml and bromocresol purple 1.6mg/ml. Plates were incubated for 7days at 37ºC. Colonies that exhibited a colour change from light green to purple were purified and identified using Mycology online (2001). Isolates (Bacteria and Dermatophytes) were maintained on Nutrient Agar (NA) and on Sabouraud Dextrose Agar (SDA) slants respectively at 4^{0}C in refrigerators for further identification.

Preparation of crude extracts. A stock concentration of 100mg/ml of solid crystal and oil from petroleum ether crude extract, chloroform, methanol crude extracts and fractions A, C, D and E from the column chromatography respectively were prepared by dissolving 1g of each crude extract in 10ml Dimethyl sulphoxide (DMSO), which was used for the susceptibility test.

Antibacterial assay. The Kirby-Bauer diffusion method on Mueller-Hinton Agar was used for the antibacterial activity for the different crude extracts. A standard concentration of bacteria strain was prepared using 0.5M McFarland standard under aseptic condition. A loopful of colony (bacteria) on a sterile inoculating loop was inoculated into a test tube containing 5ml of freshly prepared sterile physiologic saline (i.e. prepared by dispensing 8.5g of sodium chloride salt in 1000ml of distilled water). The broth was left to stand for 5-10 minutes and its turbidity compared with the 0.5M McFarland standard. A sterile non-toxic swab stick was dipped into the adjusted suspension and pressed several times on the wall of the test tube, then inoculated into the Mueller -Hinton Agar media by spreading the plates. The sterile disc was impregnated with crude extracts and placed into agar plates and allowed to stand for 15 minutes to dry. The inoculated plates were incubated at 37^{0}C for 24 hours. A control test was also carried out using dimethyl sulphoxide (DMSO) only. Antibacterial activity of the extracts was determined by measuring the Zone of inhibition. (Coyle, 2005)

Antifungal assay. This was done using the modified method of Rahman *et al*., (2001). Sabouraud Dextrose Agar (SDA) was prepared by mixing 4% of glucose agar and agar in distilled water, stirred with a magnetic stirrer to dissolve it and 3ml amount was dispensed into bijou bottles, upon sterilization (at 121°C for 15mins). Bijuo bottles were allowed to cool to 50°C and the test sample (crude extracts) of 0.1ml concentration pipetted from the stock solution into the non- solidified Sabouraud agar media and allowed to solidify at room temperature, each bijou bottle was inoculated with 4mm diameter of innocullum removed from a seven day old culture of fungi. A control test was also carried out using dimethyl sulphoxide (DMSO) only.

Determination of minimum inhibitory concentration. The minimum inhibitory concentration (MIC) of the potent crude extracts was determined using the method described by Rahman *et al.,* (2001). Various concentrations of chloroform, methanol and oil crude extracts such as 1:2, 1:4, 1:8, and 1:16, 1:32, 1:64

(i.e. 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, and 1.563mg/ml) were obtained by dilutions of the stock concentrations using DMSO. The MIC of the extracts was defined as the lowest concentration of plant extract that caused growth inhibition.

RESULTS AND DISCUSSION

The GC results of the crude extracts of *B. coriacea* showed that petroleum ether contained fifty (50) compounds (Table 1), chloroform had forty (40) compounds (Table 2), while methanol afforded ten (10) compounds (Table 3).

Table 1: Chemical composition of the petroleum ether extract (oil) fraction

S/no	Compounds	Retention Time	Area %
1	(E)-9- eicosene	3.167	0.78
2	1 , 1'- oxybis- decane,	3.945	4.25
3	2-ethylhexyl hexylester oxalic acid	4.311	3.67
4	1-ethyl-3-methyl- benzene	4.162	8.40
5	2-methyl- trans-decalin	4.368	2.93
6	1-ethyl-3, 5-dimethyl- benzene	4.523	2.14
7	Trans- decahydro- naphthalene	4.834	3.45
8	Dodecyl 2-propyl sulfurous acid,	4.946	0.63
9	O- cymene	5.026	3.58
10	Undecane	5.622	2.95
11	1-ethyl-2, 3-dimethyl- benzene	5.479	1.95
12	1- (1, 1-dimethyl ethyl)-3-methyl- benzene	5.679	0.62
13	1, 2, 4, 5- tetramethyl- benzene	5.730	0.92
14	1, 2, 3, 4- tetramethyl- benzene	5.787	0.81
15	decahydro-2-methyl- naphthalene	5.850	0.49
16	1, 2, 3-trimethyl- benzene	3.590	1.34
17	1, 2, 4-trimethyl- benzene	3.773	6.91
18	1-ethyl-2-methyl- benzene	3.813	3.88
19	1-methyldecahydronaphthalene	6.165	1.02
20	1, 2, 3, 4- tetrahydro- naphthalene	6.520	0.22
21	**1-(2, 3-dimethyl phenyl) ethanone**	**6.726**	**0.20**
22	**Azulene**	**6.938**	**0.70**
23	**Dodecane**	**7.796**	**0.26**
24	**4H- pyran-4-one (chloromethyl)-**	**9.335**	**0.04**
25	**2-methyl- naphthalene**	**9.661**	**0.24**
26	**1-methyl- naphthalene**	**10.068**	**0.14**
27	**Tridecane**	**10.474**	**0.19**
28	**2, 4-bis (1, 1-dimethyl ethyl)- phenol**	**15.858**	**0.02**
29	**1-noadecene**	**24.126**	**0.16**
30	**Methyl ester hexadecanoic acid,**	**27.062**	**0.07**
31	**Palmitoleic acid**	**27.468**	**0.62**
32	**Cis-9-hexadecanoic acid**	**27.594**	**0.13**
33	**Cyclic octaatomic sulfur**	**27.788**	**0.15**
34	**n-hexadecanoic acid**	**28.195**	**3.08**
35	**n-nonadecanol-1**	**29.036**	**0.14**
36	**(Z, Z)- 9, 12- octadecadienoic acid**	**32.000**	**4.37**
37	**Oleic acid**	**32.200**	**2.71**
38	**(E) 9- octadecenoic acid**	**32.292**	**2.01**
39	**Pentadecyl ester- trifluoroacetic acid**	**33.528**	**0.11**
40	**Ethyl ester hexadecanoic acid**	**28.727**	**0.09**
41	Methyl ester (E)- 9- octadecanoic acid	31.044	0.08
42	N- (4-bromo-n-butyl) - 2-piperidinone	37.562	0.03
43	Heptadecyl ester dichloroacetic acid	37.636	0.07
44	(Z)- 9, 17- octadecadienal	38.654	0.12
45	Bis (2- ethyl hexyl) phthalate	39.753	0.03
46	Stigmasterol	40.880	0.07
47	(Z)- 9- tricosene	41.441	0.05
48	Beta- sitosterol	44.022	0.33
49	Cycloheptamethanol	4.008	1.20
50	Octacosyl acetate	44.972	0.03

Table 2: Chemical composition of the chloroform crude extract fraction

S/no	Compounds	Retention Time	Area %	Peak No
1	2- pyrrolidinone	4.054	0.56	1
2	Tetrahdropyran	6.697	1.87	3
3	N- ((E)- butenonyl)- Oxaolidin-2-one	11.424	0.38	4
4	1, 2, 3, 4, 5, 6, 7, 8,- octahydro- Anthracene	18.490	0.76	5
5	1H- indole-3-carboxaldehyde	22.224	0.71	7
6	1-nonadecene	24.126	0.69	8
7	methyl ester Hexadecanoic acid	27.068	1.77	9
8	Palmitoleic acid	27.337	1.65	10
9	Cyclic octaatomic sulfur	27.834	10.30	11
10	n-hexadecanoic acid	28.017	11.25	12
11	ethyl ester Hexadecanoic acid	28.721	0.71	13
12	Trifluoroacetoxy hexadecane	29.030	0.60	14
13	methyl ester (Z, Z)- 9, 12-octadecadienoic acid,	30.827	2.08	15
14	methyl ester cis-13-octadecenoic acid	31.170	0.97	17
15	(Z, Z)- 9, 12-octadecadienoic acid	31.765	12.18	18
16	Oleic acid	31.977	9.70	19
17	(E)-9-octadecenoic acid	32.074	9.88	20
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18	ethyl ester 9, 12-octadecadienoic acid	32.355	1.58	21
19	Octadec-9-enoic acid	32.503	1.13	22
20	1, 19- eicosadiene	32.544	0.65	23
21	Eicosanoic acid	33.041	0.37	24
22	1-heneicosyl formate	33.516	0.39	25
23	N-(4-bromo-n-butyl)- 2-piperdinone	35.027	0.79	26
24	n-propyl 9-octadecenoate	36.572	0.40	28
25	tetradecyl- Oxirane	37.945	0.48	29
26	13- tetradecen-1-ol acetate	38.517	0.79	31
27	Glycerol 1- palmitate	38.968	0.46	33
28	Bis (2-ethylhexyl) phthalate	39.747	1.54	34
29	Cis-9-hexadecenal	42.294	1.12	36
30	n- propyl 11- octadecenoate	42.420	0.88	37
31	Beta-sitosterol	43.987	1.44	38
32	2-(4- fluorophenyl) indole	47.163	0.06	40
33	6-ethoxy-2 (3H)-benzothiazolethione	47.752	0.71	44
34	2-(2- benzoxazolyl)- Phenol	47.850	0.29	45
35	Trimyristin	48.096	1.63	46
36	2-hydroxy-1, 3- propanediyl ester Tetradecanoic acid,	48.256	0.72	49
37	Acridine-9-thiole	48.399	0.90	51
38	3-amino-8-(pyrrolidine-1-sulfonyl)- Dibenzofuran	48.657	9.32	53
39	di (3, 5-difluorophenyl) ester Fumaric acid	48.854	0.91	54
40	methyl ester (E)- (9)- octadecanoic acid	31.039	1.34	16

Table 3: Chemical composition of methanol crude extract fraction

S/no	**Compounds**	**Retention Time**	**Area %**	**Peak No**
1	Triethylmethoxy- Silane	3.207	1.17	1
2	1-methyl-pyrrolidine-2-carboxylic acid	6.268	36.07	4
3	5-Hydromethylfurfural	7.144	16.53	6
4	1-(-)-4-Hydroxy-1-methylproline oxalic acid	7.693	2.33	7
5	Hexyldecyl ester cyclohexylmethyl Sulfurous acid	10.319	1.86	9
6	2- (trimethylsilyl) thiazole	12.208	1.52	10
7	2-vinyl-9-(beta-d-ribofuranosyl) hypoxanthine	12.419	5.75	11
8	(E) 2, 3- dimethylcyclohex-2-en, oxime	12.608	2.12	12
9	Methyl ester 3,5-dicyclohexyl-4-hydroxy- Benzoic acid	48.651	9.56	13
10	5-methoxy- 2-(2, 3-dimethoxyphenyl)- 3-phenylpropionic acid	48.685	8.53	14

The mass spectra of some of these compounds indicated presence of cyclooctasulphur, (m/z 256 with base peak at m/z 64), oleic acid (m/z 282, base peak at m/z 55), 5-hydroxymethylfurfural (m/z 126, base peak 97), 1-Methyl-pyrrolidine-2-carboxylic acid (m/z 129, base peak, m/z 84), n- Hexadecanoic acid (m/z 256, base peak at m/z 60) and Methyl ester (Z, Z)-9,12- octadecadienoic acid (m/z 294 with base peak at 67). The isolation of dermatophytes gave three isolates that exhibited colour changes from

green to purple, which were subcultured for identification on Sabouraud Dextrose Agar (SDA) plates. They were identified by their colonial morphology (Table 4).

Table 4: Identification of Dermatophytes

Isolates	Morphology	Microscopy	Suspected Dermatophytes
Isolate 1	Slow growing greenish brown with suede-like surface raised and folded in the centre on SDA.	Macro conidia have smooth thin walled like surface in clusters	*Epidermaphyton floccosum*
Isolate 2	Flat, slightly raised, white to cream, suede -like to downy, with a yellow-brown to wine -red reverse.	Slender cigar like microconidia	*Tricophyton rubrum*
Isolate 3	Colonies are flat, creamy with powdery suede like surface.	Spiral hyphae, Spherical Chlamydospores present with smooth walled, multi septate Macroconidia	*Tricophyton interdigitale*

The susceptibility test carried out on the Gram-positive and Gram-negative bacteria and the dermatophytes (fungi) using the crude extracts of the seeds of the fruit of *B.coriacea* indicated that all the bacterial isolates showed resistance to the crude extracts with no observed zones of inhibition (Table 5).

Table 5: Susceptibility result of crude extracts on Bacterial isolates**.**

Isolates	Methanol extract	Chloroform extract	Solid crystal	Oil	Methanol fraction A	C	D	E
Escherichia Coli	_	_	_	_	_	_	_	_
Staphylococcus Aureus	_	_	_	_	_	_	_	_
Klebsiella Pneumonia	_	_	_	_	_	_	_	_
Proteus mirabilis	_	_	_	_	_	_	_	_
Pseudomonas aeuroginos	_	_	_	_	_	_	_	_

Key: - ++= No growth (high Susceptibility)
- = Resistant

The fungi gave positive results by responding differently to the various extracts. Isolate 1 (*E. floccosum*) was shown to be susceptible to crude extracts of methanol, oil and fraction D. Isolate 2 (*T. rubrum*) was found to be susceptible to chloroform extracts, oil and fractions A, C whereas isolate 3 (*T. interdigitale*) were found susceptible to crude extracts of methanol, chloroform, solid crystal (cyclooctasulphur), oil and fraction A (Table 6).

Table 6: Susceptibility result of crude extracts on Dermatophytes isolates.

Isolates	**Methanol extract**	**Chloroform extract**	**Solid crystal**	**Oil**	**Methanol fraction A**	**C**	**D**	**E**	
Epidermophyton floccosum	+ +	-	-	++	-		-	+ +	-
Trichophyton rubrum	-	+ +	-	+ +	+ +		+ +	-	-
Trichophyton interdigitale	+ +	+ +	+ +	+ +	+ +		-	-	-

Key:- ++ = No growth (high susceptibility)
- = Resistant

The control test (performed with DMSO) showed no inhibition on isolates. The results obtained for the MIC assay on the 3 crude extracts showed that *E. floccosum, T.rubrum, and T.interdigitale* were most susceptible to the oil (petroleum ether crude extract) by showing a wider range of susceptibility as far as 1:32 (3.125mg/ml) concentration (Table 7-9).

Table 7: Minimum inhibitory concentration of crude extracts on *E. floccosum.*

Crude Extracts/DMSO	1:2	1:4	1:8	1:16	1:32	1:64
Methanol	+ +	+ +	-	-	-	-
Oil	+ +	+ +	+ +	+ +	+ +	-

Key:- + + = No growth (high susceptibility)
Resistant

Table 8: Minimum inhibitory concentration of crude extracts on *T.rubrum.*

Crude Extracts/DMSO	1:2	1:4	1:8	1:16	1:32	1:64
Chloroform	+ +	+ +	+ +	-	-	-
Oil	+ +	+ +	+ +	+ +	+ +	-

Key:- + + = No growth (high susceptibility)
- = Resistant

Table 9: Minimum inhibitory concentration of crude extracts on *T. interdigitale*

Crude Extracts/DMSO	1:2	1:4	1:8	1:16	1:32	1:64
Chloroform	+ +	+ +	+ +	+ +	-	-
Oil	+ +	+ +	+ +	+ +	+ +	-
Methanol	+ +	+ +	+ +	+ +	-	-

Key:- + + = No growth (high susceptibility)
- = Resistant

The methanol crude extract had a poor activity on *E.floccosum* with MIC value of 1:4 (25mg/ml) concentration (Table 7) and on T.interdigitale with MIC of 1:16 (6.25mg/ml, Table 9). Also chloroform extract had MIC value of 1:8 (12.5mg/ml) concentration *on T.rubrum (Table 8*) and 1:16 (6.25mg/ml) concentration on T.interdigitale (Table 9) respectively. The GC-MS analysis of the crude extracts showed the presence of some major compounds, which contributed to the antimicrobial activity of the seeds. These include oleic acid, (Z, Z) - 9, 12- octadecadienoic acid, n-hexadecanoic acid, methyl ester (Z, Z)-9, 12-octadecadienoic acid and (E)- 9-octadecenoic acid, which are known to have antifungal potential (Seidel and Taylor, 2004, Hong-Wei *et al.*, 2014). Palmitoleic acid, 5-hydromethylfurfural, cyclooctasulphur (Ojinnaka *et al.*, 1992), 3-amino-8-(prrolidine-1-sulfonyl)-dibenzofuran, 1-ethyl-3-methyl-benzene (Sujathal *et al.*, 2011), methyl ester 3, 5-dicyclohexyl-4-hydroxy-benzoic acid and 1-methyl-pyriolidine-2-carboxylic acid, which have been shown in literature to possess antifungal and antibacterial potential were identified as constituents of crude extracts of Buchholzia coriacea. The result of present study shows that the seeds of the fruit of *Buchholzia coriacea* possess medicinal and pharmacological properties. The variety of the medicinal and pharmacological potential possessed by this plant may be traced to some of the identified compounds acting either singly or in synergy. Compounds having antimicrobial, wound healing and anti-inflammatory potential were identified. This result encourages the continuous use of the seed of *Buchholzia coriacea* in traditional medicine and also the possibility of the use of the identified constituents as lead compounds in the development of new useful drugs. The presence of the constituents might have contributed to the use of the plant extracts as antimicrobial agent in traditional medicine and in the treatment of various diseases.

Acknowledgement. The authors are grateful to A.A. Ibitoye, Central Research Laboratory, Federal University of Technology, Akure, Nigeria for running the GC-MS samples and Dr. Edwin Wosu, University of Port Harcourt herbarium, for authenticating the plant material.

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