the expected patterns of differences in phase angles with varying LD ratios in the present study, raise an interesting question. How is it that a tropical animal, not used to much variations in LD ratios (in its recent evolutionary past) can still show the same behaviour as temperate animals with respect to scaling of α but cannot do so with respect to varying the phase angles. It is interesting to speculate that scaling of α with durations of D is a simpler phenomenon not really requiring prior experience or adaptation while varying the phase angles as temperate animals do is a more complicated phenomenon which involves anticipating the onset of light or darkness. This may be dependent on prior experience or adaptation. However, this speculation is based on this single result with a tropical animal. Given the extreme paucity of information on the behaviour of tropical animals under varying LD ratios and the unusual results obtained in this study, more work along these lines on other tropical animals, diurnal and nocturnal, is sorely needed.

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Potent amoebicides from plant extracts – An *in vitro* assessment with the gum-oleo-resin of *Commiphora wightii*

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The medicinal value of *Commiphora wightii* has been believed by tribals to be mainly due to its yield of guggulipid, which has been scientifically shown to have hypochloesteromic, anti-septic, anti-pathogenic, anti-parasitic properties. It is also used for non-specific diarrhoea and dysentery. Amoebic dysentery is a common disorder of a large number of people in the tropics. In our studies we have reviewed the active principles of most known anti-amoebic plants. Further, we have tested the crude extracts of oleo-gum-resin obtained from *C. wightii* against *E. histolytica* NIH 200 using microdilution technique. They were found to be comparable with quassinoids; Ailanthinone and Bruceantin. The need for linkages between chemical characterization with established *in vitro* techniques is demonstrated.

AMOEBIASIS by *Entamoeba histolytica* is an important cause of dysentery. Recent global estimates indicate the increasing trend from 480 million people (excluding China) annually suffering from amoebiasis[1]. The search for herbs and medicines for this scourge, from all possible sources is an ongoing exercise. Many natural products of plant origin are an important source of biologically active compounds and have potential for the development of novel antiprotozoal drugs as studied by *in vivo* and *in vitro* experimentations[2]. Table 1 shows known anti-amoebic plants studied since the last decade for their active fractions and principles.

Commiphora wightii (Arnott) Bhand, (Burseraceae) is a small tree or large-sized shrub which produces a gum 'guggal' believed to have high medicinal value. It is commonly known as 'Indian bdellium' or 'guggal' in India[3]. It is found in the arid rocky tracts of Rajasthan, Gujarat, Karnataka and Maharashtra states of India; Sindh and Baluchistan states of Pakistan; Bangladesh and Arabia[4]. The trunk is knotty, outer bark comes off in rough flakes leaving an inner layer which is bright, shining and peels off in thin rolls like paper. The latex oozes out through wounds or cuts as a yellow fluid which hardens to form a golden brown, yellow or reddish brown oleo-gum-resin. Guggal gum is a mixture of 61% resin, 29.3% water, 0.6% volatile oil and 3.2% foreign matter[3]. Guggal gum is known for its therapeutic properties in various ailments, particularly arthritis, many vascular and neurological complications, hypercholesteremic conditions[5], rheumatism and possesses anti-inflammatory activity[6], in cure of ischaemic heart disease, obesity, neurological disorders, ills of syphyllitic nature[7], scrofulous infections, urinary disorders and a few skin diseases[4]. Its essential oil also possess antibacterial, antifungal and antihelminthic activity.

As one of the ingredients in 'Arogya Vardhini Bati', an Ayurvedic drug, *Commiphora* is used for the treatments of diarrhoea and dysentery in man and its efficacy has been tested both *in vitro* and *in vivo*[8]. Alcohol extract of its oleo-gum-resin was tested *in vitro* against axenic cultures of *E. histolytica* NIH 200 but proved less effective than *Curcuma zedoaria*[9]. The most optimal comparisons of other such plants which were similarly studied for their active principles are *Brucea javanica*, *B. antidysenterica* and *Simarouba amara* with quassinoids; Bruceantin and Ailanthinone as their active compounds.

Table 1. Different plants possessing antiamoebic potential along with their active fractions

Plant sps.	Family	Part used	Fraction/extract	Remarks and reference no.
Allium sativum	Liliaceae	Cloves	Crushed cloves	Allicin inhibits the growth of axenically cultured *E. histolytica* at 30 µg/ml provided cysteine was not present in the medium. The activity of gossypol, a polyphenolice compound was 11 and 39 times greater than those of metronidazole and emetine respectively[2].
Gossypium herbacium	Malvaceae	Seed	Oil	
Brucea javanica	Simaroubaceae	Fruit	Petroleum ether fraction, aqueous fraction, chloroform fraction	A little activity was present in petroleum ether fraction of either species but chloroform fraction of the two was highly active[12].
Simarouba amara	Simaroubaceae	Stem	Chloroform fraction	
Brucea antidysenterica	Simaroubaceae	Fruit	Chloroform fraction	Rates of amoeba killings by metronidazole and bruceantin, a quasssinoid, were similar at 16 to 30 fold lower water conc. of the latter[13].
Strychnos gossweileri	Loganiaceae	Root bark	Alcoholic extract	Diploceline, a quaternary alkaloid isolated is active at 50 µg/ml on *E. histolytica*[14].
Euphorbia hirta	Euphorbiaceae	Whole plant	Ethylacetate fraction	Quercitrin, a flavonoid was active as antidiarrhoeic from doses of 25 mg/kg onwards[15].
Alstonia angustifolia	Apocynaceae	Root	Methanol extract	Macrocarpamine, an alkaloid was found to be the most potential antiamoebic compound but 4 times less potent than the standard drug, emetine[16].
Triclisia sps.	Menispermaceae	Wood	Alcoholic extract	Armoline, isotrilobine and insularine were the most active bisbenzylisoquinoline alkaloids having IC_{50} in the range of 5–11 µM (ref. 17).
Holarrhena antidysenterica	Apocynaceae	Fruit	Alcoholic extract	Conamine, holarrhimine, conkurchine, etc. were the most active alkaloids[18].
Strychnos sps.	Loganiaceae	Stem	Alcoholic fraction	Usambarine and usambarensine were the most selective alkaloids. The latter possesses activities similar to those of emetine and metronidazole[19].

In the present study an attempt has been made to test the *in vitro* anti-amoebic potential of various crude extracts of *C. wightii* using the microdilution technique. Gum resin was collected from Ajmer district (Rajasthan) and air-dried. Gum-resin (40 g) was taken, ground and extracted with 300 ml of ethyl alcohol for 10 days at 60–80°C using soxhlet apparatus. Ethanol containing the compounds was filtered and the filtrate heated at a relatively low temperature to evaporate ethyl alcohol. The ethanol soluble portion contributes about 75% of the gum resin (28.1 g). This was followed by cold extraction using petroleum ether (60–80°C). About 150 ml of petroleum ether was added to the left compound and kept for 2 days without heating so as to enable the oils and other compounds to solubilize in it. This was heated for about half-an-hour at 30°C, filtered and the filtrate was heated up to 30°C till petroleum ether evaporated. The residue was extracted with about 200 ml of chloroform and heated at 40–50°C for half-an-hour. It was cooled down, filtered and concentrated after heating. The leftover gum in the extractor, from which compounds were extracted initially using ethanol, was taken out in a conical flask, water was added to it and kept overnight. Next day it was filtered and the filtrate was heated to evaporate water. Thus, the three extracts, viz. 9.2 g of petroleum ether (yield 32.7%, w/w), 15.3 g of chloroform (yield 54.4%, w/w) and 3.6 g of aqueous (yield 12.7%, w/w) were ready for testing. *In vitro* testing against *E. histolytica* was carried out thrice in triplicate cultures using microdilution technique. Ethanol (50 µl) was added to 10 mg of each extract followed by 950 µl of fresh culture medium. They were further diluted twice with culture medium so as to obtain a concentration of 0.1 mg/ml. These were considered as stock solutions and from them 6 serial dilutions were prepared, as shown in Table 2, using fresh culture medium (final volume, 170 µl).

Each microtitre plate included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae), test wells (culture medium plus crude extracts) and a blank (culture medium only). Amoebae cell suspension 170 µl was added to the test and control wells in the plates so that the wells were completely filled (total volume 340 µl). Plates were sealed with expanded polystyrene, secured with tape and placed in an incubator at 37°C. After 48 h of incubation, inhibition of growth was assessed by measuring OD with an ELISA reader at 540 nm. When compared against the standard drug metronidazole, the chloroform extract was found twice as promising as metronidazole in its activity (Figure 1). The high content (8.24%) of chloroform extract which was taken from the original 40 g of the gum resin, and also its comparably higher activity, together makes the chloroform extract an important target for further characterization. In a separate study done, the chloroform extract of *C. wightii* was shown to contain guggulsterol I–V, guggulsterones E and guggulsterones Z[19]. Table 2 shows comparable effectiveness

Table 2. Relative effectiveness of various extracts and metronidazole (standard drug)

Conc. of extract/drug (μg/ml)	Log of conc.	Inhibition by metronidazole extract (%)	Inhibition by chloroform extract (%)	Inhibition by aqueous extract (%)	Inhibition by petroleum ether extract (%)
0.125	–0.9030	23.86	43.79	43.09	31.80
0.25	–0.6020	38.59	52.19	51.01	38.48
0.5	–0.3010	53.33	60.59	58.94	45.16
1	0	68.07	68.98	66.86	51.84
2	0.3010	82.81	77.38	74.79	58.52
4	0.6020	97.54	85.78	82.71	65.20

IC_{50} for the standard drug, metronidazole = 0.43 μg/ml.
IC_{50} for chloroform extract = 0.22 μg/ml.
IC_{50} for aqueous extract = 0.24 μg/ml.
IC_{50} for petroleum ether extract = 0.88 μg/ml.

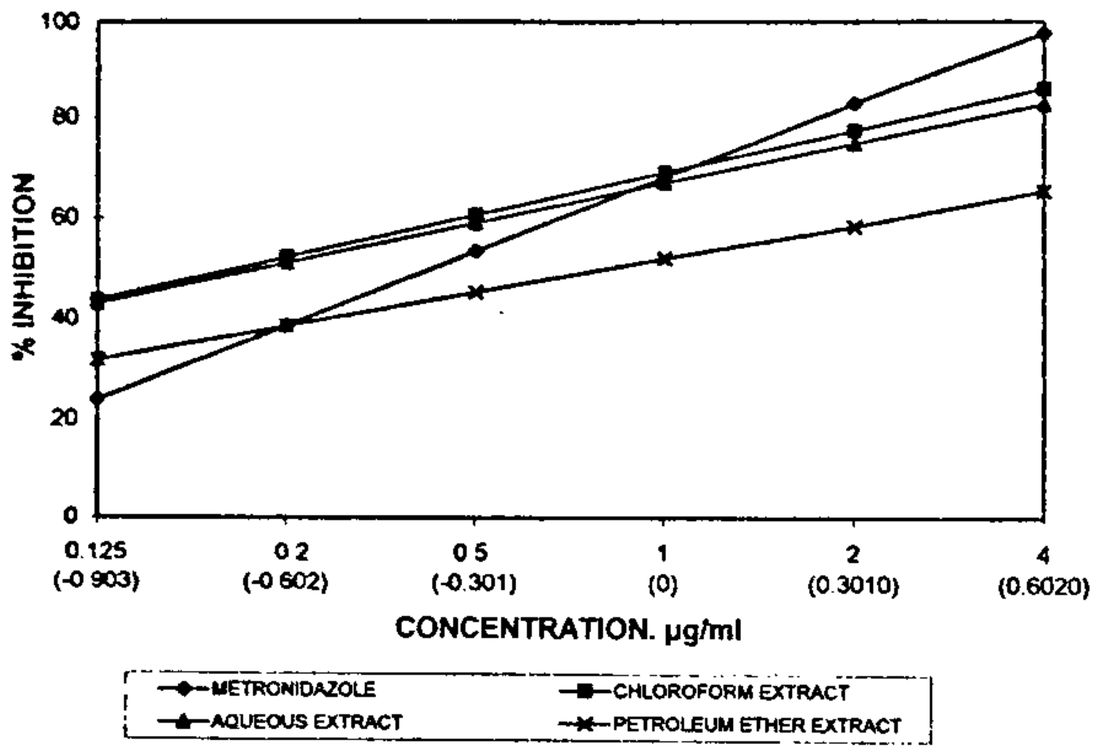

Figure 1. Activity of standard drug metronidazole, chloroform extract, petroleum ether extract and aqueous extract obtained from gum-oleo-resin of *Commiphora wightii* against *Entamoeba histolytica* (NIH 200) *in vitro*. (Log of the corresponding *X*-axis values are given within the brackets).

of three crude extracts of *C. wightii* and the standard drug metronidazole.

The active principles from *C. wightii* have been classified into four fractions, the oleo fraction containing myrcene compounds, gum fractions A and B containing sugars and resin fraction containing sterols[3]. The ethyl acetate soluble portion constitutes about 45% of the gum resin, the hexane phase material constitutes about 9% of the gum resin and consists essentially of the diterpenoids, besides a small percentage of cholesterol and other unidentified compounds. Material contained in the benzene phase constitutes about 14% of the gum resin[20]. Mukolol, a new diterpene alcohol, had been determined and isolated from the gum resin of *C. wightii*[21].

Since alkaloids and quassinoids of *C. wightii* have not been studied extensively by these standard techniques, an effort should be made in this direction. The arduous and longstanding sporadic attempts in defining the principles of trying to find plant extract efficacies are in many ways incomplete. Some of these principles can even include the subjective but to some extent justified beliefs of tribals and non-allopathic methods of treatments. They need to be reviewed and correlated with better defined standards and refined further to include biologically active fraction with their characteristics.

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