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Pharmacognostic Studies and Chromatographic Analysis of the Gum of *Anacardium occidentale* L (Anacardiaceae)

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ABSTRACT

Microscopy of the gum revealed various shapes and sizes which disintegrated within a short time (3-5 minutes). They have thick walls, somewhat cracked and striated and also seen as translucent masses. Some were observed to have open fissures. However, the purified ones obtained from the precipitates depicted no open fissures, striations or cracks. Macroscopy revealed that *A.occidentale* gum has irregular shapes, tasteless, odourless, very coarse texture for the crude to fine coarse for the purified gum. It is yellowish brown colour for the crude to whitish milk for the purified gum. On the other hand, the gum arabic has a bland mucilaginous taste, odourless, varying shapes and sizes with a milky colour. This shows that the plant gum has features similar to available pharmaceutical gums and as such a viable pharmaceutical material and also these features are useful for the preparation of monograph of the plant. Paper and Thin Layer Chromatographic analyses of the carbohydrates in both the gums revealed the presence of sugars such as xylose, arabinose, galactose and glucose. Butanol-Acetic acid- Water (BAW) 4:1:5; Butanol-Ethanol-Water (BEW) 4:1:2.2; and Butanol-Acetic acid-Ether-Water (BAEW) 9:6:3:1 were used as solvents systems by ascending technique and sprayed with Aniline phthalate for visualization.

INTRODUCTION

Gums are natural plants hydrocolloids that may be classified as anionic or non-ionic polysaccharides or salts of polysaccharides. They are translucent, amorphous substances that are frequently produced in higher plants as protective agents after injury. Thus, they are the abnormal products of plants metabolism (Kokate *et al*, 2002). Gums are also considered to be pathological products formed upon injury of the plant or owing to unfavourable conditions such as drought by a breakdown of cell walls (extra cellular formation- gummosis) (Evans, 2002; Kokate *et al*, 2002). Comparative studies using samples of cashew gum obtained from different geographical sources have been reported to show significant variations in compositions and properties linked with climatic conditions (Lima *et al*, 2002). Gums are heterogeneous polyuronides which on hydrolysis, they yield sugars such as arabinose, galactose, glucose, mannose, xylose and various uronic acids (Kokate *et al*, 2002). In most gums, the polyuronides of mixed composition are formed by glycosidic linkages and various sugar molecules (Wallis, 1967). The cashew gum was determined by Mothe and Rao (1999) to be acidic to litmus paper which is in agreement with range of cashew gum mucilage

Gums consisting of linear polymers are less soluble than those with branched constituents, and linear hydrocolloids yield solutions with greater viscosity. Plants exudates have been the traditional gums for pharmaceuticals purposes and they still find significant application; however preparation of these gums is labour intensive and carries a premium price and their use will probably continue to decline. Marine gums are widely used as utility gums at the present time, and their competitive positions appear stable (Kokate *et al*, 2002 and Tyler *et al*, 1988).

The chromatographic methods of analysis provide information on the homogeneity, molecular size and structure of a carbohydrate and gives useful information especially R_F values which are used in the identification of the compound desired. Pharmacopoeias are increasingly employing thin-layer chromatography as a means for assessing quality and purity. The RF value (rate of flow, i.e. distance moved by solute divided by distance moved by solvent front) of a compound, determined under

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specified conditions, is characteristic and can be used as an aid to identity. The R_F values vary from 0.0 to 1.00. Quantitative extracts of crude drugs are prepared and compared chromatographically with the standard reference solutions of the known constituents (Trease and Evans, 1983). The method of separation is also useful in the isolation of carbohydrates and their derivatives. Paula and Rodrigues (1995) also reported the presence of arabinose, glucose, rhamnose, mannose and glucuronic acid appearing as terminal residues in the polysaccharide of *A. occidentale* gum

Gums find diverse applications in pharmacy as tablet binders, emulsifiers, gelatine agents, suspending agents, stabilizers and thickeners. They are also ingredients in dental and other adhesives and in bulk laxatives (Tyler *et al,* 1988). Zakaria and Rahman (1996) observed that differences in gum sources seem to influence the pH and the viscosity of the gum mucilage.

MATERIALS AND METHODS

Following the identification of the *A. occidentale,* the gum was collected upon wounding or injuring the bark using a sterile beaker container. The gum was allowed to air dry under the shade and some brownish to red particles suspected to be cork or fragments of bark were mechanically removed. The hardened cakes were size reduced to fine powder by the use of pestle and mortar. About 500g of the gum powder was dissolved in about 1 litre of hot water and the resultant solution was strained to free it from insoluble matter (organic matter) by filtering, through a clean piece of linen cloth. The gum from the filterate was then extracted or precipitated using the method of Karawya *et al* (1971) for gum purification and extraction. The gum was extracted severally with 95% alcohol and finally washed and dried in the oven at a temperature of 40^0C for at least 3hours and kept in an air tight container for further use.

Detailed macroscopical and microscopical studies of the cashew gum with respect to gum Arabic were carried out. Following the acid hydrolysis, the chromatographic analysis of the gum was done using paper and Thin layer chromatography by calculating the R_F values and compared with the reference sugars. Butanol-Acetic acid- Water (BAW) 4:1:5; Butanol-Ethanol-Water (BEW) 4:1:2.2; and Butanol-Acetic acid-Ether-Water (BAEW) 9:6:3:1 were used as solvents systems by ascending technique and sprayed with Aniline phthalate for visualization.

RESULTS AND DISCUSSIONS

From the physical examination of the gum, it can be deduced that it has varying shapes and sizes, odourless, tasteless. The crude gum was seen to be yellowish brown in colour while the purified or precipitated gum is whitish to milk in colour. Under the microscope, the crude gum appeared as large masses which dissolve when cleared with a chloral hydrate as a clearing agent. The precipitated gum on the other hand showed irregular with varying shapes and sizes. They have thick walls, somewhat cracked and striated and also seen as translucent masses. Some were observed to have open fissures (figure 1). However, the purified ones obtained from the precipitates depicted no open fissures, striations or cracks (figure 2).

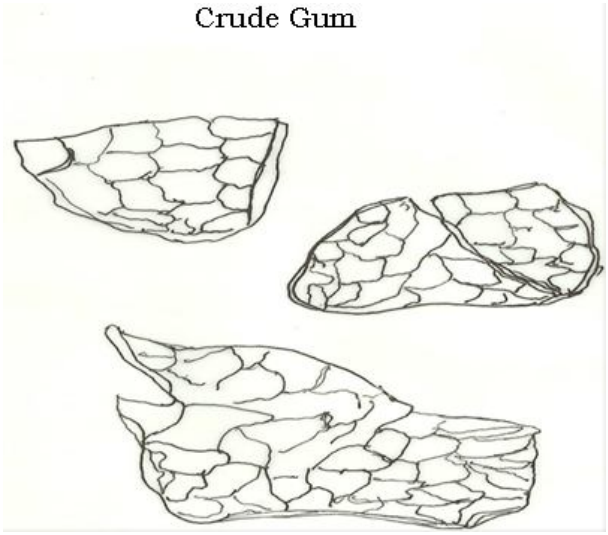

Fig. 1: Microscopical presentation of crude *A. occidentale* gum fragments.

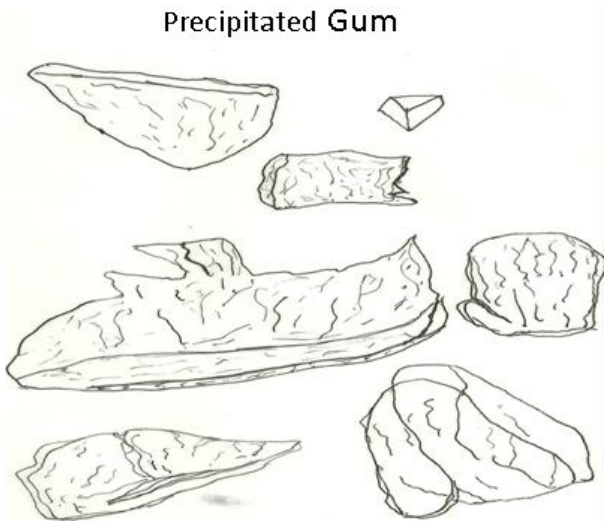

Fig. 2: The microscopical presentation of precipitated *A. occidentale* gum.

The microscopy of the gum Arabic also shows similar features like that of the precipitated cashew gum as in figure 2. The fissures/cracks have reduced due to partial purification. The observed features disintegrated within 3-5 minutes when mounted with dilute glycerol. Negligible, limited fibres were also seen. The macroscopical and microscopical feature of gum Arabic is much alike to that of cashew gum. The rapid disintegration of the *A. occidentale* gum may probably suggest its low stability property. Based on the R_F values of the various reference sugars and compared with the test gum sample, it can be inferred that the later have xylose, glucose, arabinose and galactose sugars as revealed on both the paper and thin layer chromatography. The TLC in BEW revealed that the R_F value of the gum sample (0.58) closely corresponded to that of glucose (0.55). Similarly, the TLC in BAEW solvent system indicated the R_F value of the gum (0.36) closely related to galactose (0.37). These are similar to earlier

reports by Bose and Biswas (2002). It was also observed that in PC, the R_F values of the gum sample, 0.32 and 0.15 closely related to arabinose (0.32), galactose (0.17) and glucose (0.16) in the solvent system, BEW (4:1:2.2), after spraying with aniline phthalate and heated at 105^0C for 10minutes. This is in accordance to Murthy and Yadava (1972) who reported that hydrolysis of the polysaccharide of *A. occidentale* gum yielded arabinose, galactose, glucose, rhamnose.

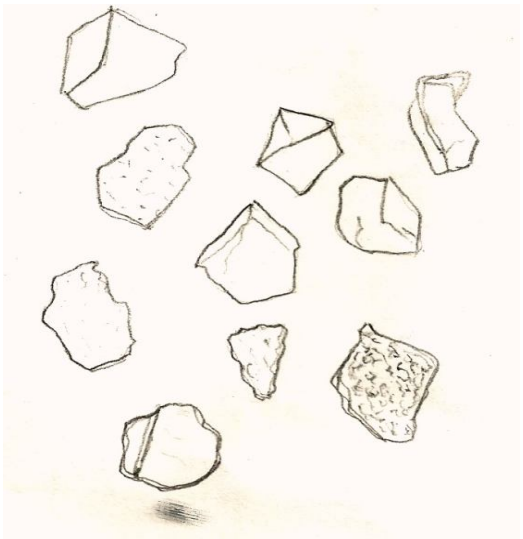

Fig. 3: microscopical presentation of gum arabic.

Table 1: Summary of R_F Values on Thin Layer Chromatography (TLC).

Reference Sugars	Solvent systems (TLC)	
	BAEW (9:6:3:1)	BEW (4:1:2.2)
Galactose	0.37	0.48
Glucose	0.46	0.55
Fructose	0.52	0.47
Lactose	0.27	0.29
Arabinose	0.48	0.54
Xylose	0.55	0.67
Sucrose	0.00	0.00
Sample gum		
	0.18	0.17
	0.36	0.38
	0.67	0.58
	0.84	0.78

BAEW = Butanol-Acetic acid-Ether-Water and BEW = Butanol-Ethanol-Water

The *A. occidentale* gum did not resolve well in Butanol-Ethanol-Water (BEW) 4:1:2.2 as the solvent system compared with Butanol-Acetic acid-Water (4:1:5) or in Butanol-Acetic acid-Ether-Water (BAEW) 9:6:3:1 because of the solvent systems polarity. Thus BEW (4:1:2.2) is less polar than BAW (4:1:5). The solvent systems used in the order of increasing polarity are as follows; BAW > BEW > BAEW. In conclusion, the macroscopical and microscopical features of *Anacardium occidentale* gum can be used to characterize it from other gums. This gum may also be modified to replace the other known official gums like gum arabic.

Table 2: Summary of R_F Values in three different solvent systems on paper chromatography (pc)

Reference Sugars	BAW (4:1:5)	BAEW (9:6:3:1)	BEW (4:1:2:2)
Arabinose	0.31	0.32	0.32
Fructose	0.26	0.24	0.24
Galactose	0.23	0.24	0.17
Glucose	0.26	0.27	0.16
Lactose	0.12	0.17	0
Sucrose	0	0	0
Xylose	0.30	0.30	0.27
Sample gum			
	0.29	0.22	0.15
	0.19	0.30	0.23
	0.28	0.27	0.32
	0.41	0.51	0.42

BAW = Butanol-Acetic Acid-Water, BAEW = Butanol-Acetic acid-Ether-Water and BEW = Butanol-Ethanol-Water

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