Journal of Medicinal Herbs

journal homepage:**www.jhd.iaushk.ac.ir**

Thin layer chromatographic profiling and phytochemical analysis of hexane, ethyl acetate, n-butanol and aqueous fractions of *Bombax costatum* Pellgr. and Vuillet stem bark

Nuhu Mohammed[*1], **Jamilu Yau**[2], **Aliyu Muhammad Musa**[3]

[1]*Department of Clinical Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria;*
***Email:** *nmohammed@abu.edu.ng*

[2]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria;*
[3]*Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *March 29th2024*
Accepted *June 29th2024*

Key words:
- ✓ *Bombax costatum*
- ✓ *chromatography*
- ✓ *phytochemical*
- ✓ *zaria*

ABSTRACT

Background & Aim: *Bombax costatum* belongs to the family *Bombacaceae*. It is an important medicinal plant commonly found in savanna zones of West Africa. The plant is used in the treatment of liver diseases, epilepsy, malaria and microbial infections. This study was aimed to investigate the phytochemical compounds present in various fractions of stem bark of the plant.

Experimental: Thin layer chromatographic profiling and phytochemical analysis of the hexane, ethyl acetate, *n*-butanol and residual aqueous fraction were carried out to explore the scientific basis for its ethno-medicinal value.

Results: The results showed the presence of tannins and alkaloids in ethyl acetate, *n*-butanol and residual aqueous fractions. Flavonoid was present in only the ethyl acetate fraction. Triterpenes and steroids were found only in the hexane and ethyl acetate fractions while saponin was only present in the *n*-butanol and residual aqueous fractions of *B. costatum* stem bark. Anthraquinone was absent in all the fractions of stem bark.

Recommended applications/industries: The presence of these phytochemicals supports the traditional use of *B. costatum* plant in the treatment of various diseases.

1. Introduction

Bombax costatum Pellgr. EtVuillet is a member of the family *Bombacaceae*. It is a valuable medicinal tree found mostly in the savanna zones of West Africa. It can reach a height of 5 - 15 meters in its natural habitat. It is called red-flowered silk cotton tree in English and in Nigerian languages as "*Kuriya"* and"*Kutukpaci*"(in Hausa and in Nupe, respectively). The stem bark (Mohammed *et al.,* 2023) and calyx (Eugene *et al.,* 2018) of *B. costatum* were reported to possess good *in vitro* antioxidant activity. A bath in an extract of the stem bark is used in the treatment of mental illness (Oyen, 2011). Ethanol stem bark extract of the plant was reported to possess anticonvulsant activity (Nazifi *et al.,* 2020) while the methanol stem bark extract was reported to possess hepatoprotective (Mohammed *et al.,* 2018) and anti-hepatofibrotic (Mohammed *et al.,* 2023).

In this study, the phytochemical compounds present in various fractions of stem bark of the plant was investigated.

2. Materials and Methods

2.1. Sample collection

The plant *B. costatum* was identified and collected from Hanwa area of Sabon Gari local Government

Area of Kaduna State in the month of July and subsequently authenticated by a Taxomist Mallam Namadi Sunusi, of the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria. The plant was compared with the voucher specimen in the herbarium and a specimen number (1211) was assigned to it for future reference.

2.2. Extraction and successive fractionation of plant material

The stem bark of *B. costatum* plant was cleaned and air dried under shade with intermittent weighing until a constant weight was obtained. It was then mechanically pulverized using mortar and pestle and stored in air-tight containers. Methanol extract of *B. costatum* stem bark was prepared by subjecting about 11 kg of the powdered stem bark to maceration using 40 litres of 70% v/v methanol (to prevent bacterial contamination) for 72 hours. The mixture was intermittently stirred during the 72 hours period and then filtered using Whatman filter paper No. 1. The filtrate was concentrated to dryness over a water bath maintained at 50 °C to produce dark brown residue.

Fractionation of the methanol extract was conducted according to the methods previously described (Gandi *et al.*, 2003; Leila *et al.*, 2007) with some modification in the choice of primary solvent (water) and partitioning solvents (hexane, chloroform, ethyl acetate and n-butanol). For this purpose, 160 g of methanol stem bark extract of *B. costatum* was subjected to liquid-liquid partitioning sessions. For each session, 92 g of the extract was dissolved in 500 mL of distilled water and exhaustively partitioned (3 times) sequentially with 500 mL each of hexane, chloroform, ethyl acetate and *n*-butanol. For each solvent, the mixture was allowed to stand for 30 min in the separating funnel until a fine separation line appeared. The solvent fractions and the residual aqueous fraction were evaporated to dryness over a water bath maintained at 50ºC to obtain their corresponding fractions.

2.3. Preliminary phytochemical screening

The presence of tannins, alkaloids, flavonoids, glycosides (reducing sugars), tannins, carbohydrates, anthraquinones, sterols and saponins were tested in methanol stem bark extract of *B. costatum* and its fractions by simple qualitative methods (Trease and Evans, 1996).

2.3.1. Test for alkaloids

Five hundred milligrams of the crude extract and its fractions were added to 5 ml of 1% aqueous HCl and stirred in different test tubes. The mixtures were warmed on a steam bath and filtered. A few drops of Dragendorff‘s reagent was added. A yellow-brown precipitate indicates presence of alkaloid.

2.3.2. Test for flavonoids (Shinoda Test)

Five hundred milligrams of the crude extract and its fractions were dissolved in 2 ml of 50% methanol in different test tubes. Metallic magnesium chips and few drops of concentrated hydrochloric acid were added to each test tube. A presence of red color indicates presence of flavonoids.

2.3.3. Test for saponins glycoside

About 10 mL distilled water was added to 0.5 g of each sample and was shaken vigorously in a test tube for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 min. A honeycomb froth that persists for 10-15 min indicates presence of saponins.

Test for tannins (Ferric chloride test): To 0.5 g of the extract and its fractions, 3-5 drops of ferric chloride solution was added. A greenish-black precipited indicates presence of condensed tannins.

2.3.4. Test for anthraquinones

To 0.5 g of each sample in a dry test tube, 5 mL of chloroform was added and shaken for at least 5 minutes. This was filtered and the filterate shaken with equal volume of 10% ammonia, bright pink color in the aqueous (upper) layer indicates the presence of free anthraquinones.

2.3.5. Test for carbohydrates (Molisch Test)

To 0.5 g of each sample in a test tube, few drops of Molisch reagent was added followed by concentrated sulphuric acid down the side of the tube to form a lower layer. A reddish colored ring at the interphase indicates the presence of carbohydrates.

2.3.6. Test for cardiac glycosides (Keller-killiani Test)

Five hundred milligrams of each sample was dissolved in 1 ml of glacial acetic acid containing traces of ferric chloride solution in a test tube. This was then transferred into a dry test tube and 1 mL of concentrated sulphuric acid was added down the side of

the test tube to form a lower layer at the bottom. A pale green color in the upper acetic acid layer indicates the presence of cardiac glycosides.

2.3.7. Test for triterpenes (Liebermann-Bucchard Test)

Five hundred milligrams of each sample was dissolved in 5ml of methanol and then filtered. The filtrate was evaporated to dryness at 45 °C on water bath. The residue was shaken with chloroform and filtered into a clean and dry test tube. 2 mL of acetic acid anhydride was added to the filtrate and shaken. Concentrated Sulphuric acid (1 mL) was added carefully down the side of the tube to form a lower layer. The appearance of a brownish-red or violet ring at the zone of contact of the two liquids and the upper layer turning green indicates the presence of triterpene.

2.4. Thin layer chromatographic profiling and phytochemical screening of fractions of B. costatum

Silica gel coated TLC plates measuring 20 × 20 cm were used for the analysis. The hexane, ethyl acetate, butanol and residual aqueous fractions of *B. costatum* were individually dissolved in methanol. A capillary tube was used to spot each sample on different plates about 1.0 cm from the lower edge of each plate and were dried with an air dryer. Each plate was then lowered into a small chromatographic tank containing the suitable solvent system (Butanol: Acetic acid: Water 10:1:1 for butanol and aqueous fractions; Hexane: Ethyl acetate 9:1 for hexane fraction; Hexane: Ethyl acetate 7:3 for ethylacetate fraction). The tank was covered with a glass lid. The solvent was allowed to ascend until the solvent front was about ¾ of the length of the TLC plate. The TLC plates were removed and dried with air dryer. Developed plates were viewed under daylight and UV at 254 nm to identify the fluorescingspot. The strips were then sprayed with general reagent (vanillin inH_2S0_4) and specific reagents ($FeCl_3$ for tannins, $AlCl_3$ for flavonoid, Borntrager's for anthraquinones, Dragendorff for alkaloid, Liebermann-Buchard for triterpenes and steroids) and heated at 110 °C for 5 seconds where applicable forvisibility of fluorescent bands. The number of spots and colour reaction for each plate was noted (Wahab *et al.*, 2010).

3. Results and discussion

The stem bark of *B. costatum* is widely used in the treatment of various diseases affecting humans. In this study, phytochemical screening of fractions of *B. costatum* showed the presence of alkaloids, flavonoids, tannins, carbohydrate, cardiac glycosides, triterpenes and saponins (Table 1).

Table 1. Phytochemical constituents of fractions of *Bombax costatum*stem bark.

Phytoconstituent	Hexane fraction	Ethyl acetate Fraction	n-Butanol fraction	Aqueous fraction
Flavonoids	-	+	-	-
Saponins	-	-	+	+
Tannins	-	+	+	+
Anthraquinones	-	-	-	-
Carbohydrates	-	+	+	+
Triterpenes and steroids	+	+	-	-
Alkaloids	-	+	+	+

+ = Present, - = Absent

TLC is a chromatographic technique that can be used for identification of the bioactive compound.It can also be used to determine the phytochemical constituents present in plant extract (Wahab *et al.*, 2010; Randhawa *et al.*, 2015; Sonam *et al.*, 2017).

Thin layer chromatographic profile of ethyl acetate (E), hexane (H), butanol (B) and residual aqueous (A) fractions of *B. costatum* eluted in hexane: ethyl acetate (9:1) and sprayed with vanillin in H_2SO_4 reagent showed some major spots (Fig.1) indicating the presence of bioactive secondary metabolites.

Figure 1. TLC profile of fractions of *Bombax costatum* when sprayed with vanillin in H_2SO_4 reagent.
B=n-Butanol fraction, A=Residual aqueous fraction, E=Ethyl acetate fraction, H=Hexane fraction

The TLC chromatographic analysis of ethyl acetate fraction of *B. costatum* eluted in hexane: ethyl acetate (7:3) showed absence of bright pink spot confirming the absence of anthraquinones as shown in Figure 2. Additionally, TLC chromatographic analysis of butanol (B) and residual aqueous fraction of *B. costatum* (A) eluted in butanol: acetic acid: water (10:1:1) and sprayed with Bontreger's reagent also showed absence of anthraquinones (Fig. 2).

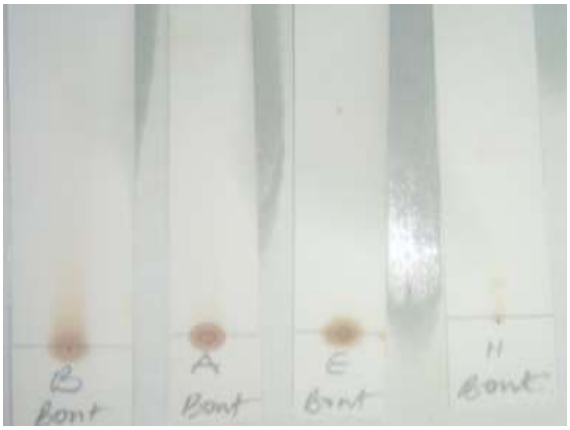

Figure 2. TLC profile of fractions of *Bombax costatum* when sprayed with Bontreger's reagent.
B=n-Butanol fraction, A=Residual aqueous fraction, E=Ethyl acetate fraction, H=Hexane fraction

However, TLC chromatographic analysis of ethyl acetate fraction of *B. costatum* eluted in hexane: ethyl acetate (7:3) and sprayed with Dragendorff reagent showed orange spot on the plate confirming the presence of alkaloids. Orange spots were also observedon the TLC plate of butanol (E) and residual aqueous fraction of *B. costatum* (A) using butanol: acetic acid: water (10:1:1) as solvent system (Fig. 3).

Figure 3. TLC profile of fractions of *Bombax costatum* when sprayed with Dragendorff reagent.
B=n-Butanol fraction, A=Residual aqueous fraction, E=Ethyl acetate fraction, H=Hexane fraction.

TLC chromatographic analysis of hexane fraction of *B. costatum* eluted in hexane: ethyl acetate (9:1) and sprayed with Liebermann-Buchard reagent showed blue-green spot confirming the presence of triterpenes and steroid. In addition, TLC chromatographic analysis of ethyl acetate fraction of *B. costatum* eluted in hexane: ethyl acetate (7:3) and sprayed with Liebermann-Buchard reagent also showed the presence of triterpenes and steroid (Fig. 4).

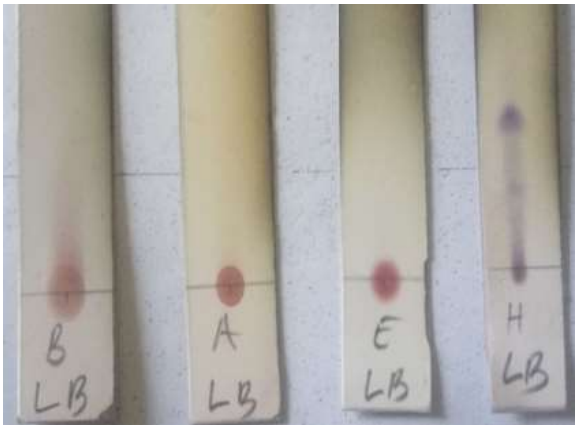

Figure 4. TLC profile of fractions of *Bombax costatum* when sprayed with Liebermann-Buchard reagent.
B=n-Butanol fraction, A=Residual aqueous fraction, E=Ethyl acetate fraction, H=Hexane fraction.

TLC chromatographic analysis of ethyl acetate (E) fraction of *B. costatum* eluted in hexane: ethyl acetate (7:3), sprayed with $AlCl_3$ and viewed under ultraviolet light also showed fluorescing spot confirming the presence of flavonoids. This test was negative for hexane, butanol and residual aqueous fraction of *B. costatum* (Fig. 5).

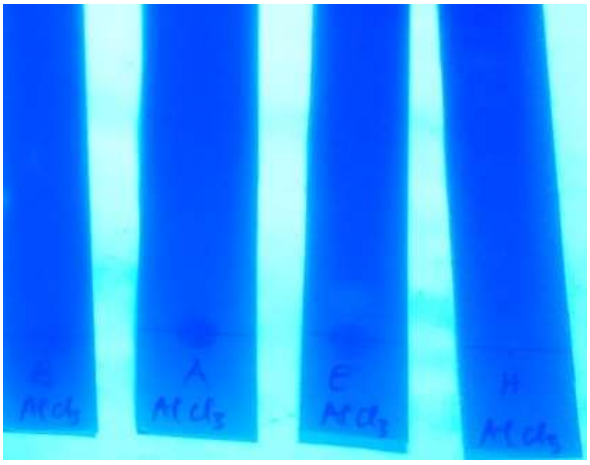

Figure 5. TLC profile of fractions of *Bombax costatum*when sprayed with $AlCl_3$ reagent.
B=n-Butanol fraction, A=Residual aqueous fraction, E=Ethyl acetate fraction, H=Hexane fraction.

Dark green spot was observed on TLC chromatographic analysis of ethyl acetate fraction of *B. costatum* eluted in hexane: ethyl acetate (7:3) after spraying with $FeCl_3$ reagent. The dark green spotconfirms the presence of tannins. Tannins were also observed to be present on TLC chromatographic analysis of butanol (B) and residual aqueous fraction of *B. costatum* (A) using butanol: acetic acid: water (10:1:1) as solvent system (Fig. 6).

Figure 6. TLC profile of fractions of *Bombax costatum* when sprayed with $FeCl_3$ reagent.
B=n-Butanol fraction, A=Residual aqueous fraction, E=Ethyl acetate fraction, H=Hexane fraction.

Most of the phytochemicals namely alkaloids, flavonoids, tannins, carbohydrates, triterpenes and steroids were found in the ethyl acetate fraction of *B. costatum*. This finding corroborates with the findings of a previous study (Mohammed *et al.*, 2020). Antioxidants are essential in the prevention of oxidative stress-related diseases and in reducing the burden associated with these diseases (Laurence *et al.*, 2008). Secondary metabolites such as flavonoids, tannins, alkaloids and saponins found in fractions of *B. costatum* stem bark are known to possess antioxidant propertiesvia free radical scavenging effect (Panche *et al.*, 2016). Phenolic compounds such as flavonoids were in addition reported to possess anti-inflammatory, antiallergic, antitumor and antimicrobial activities (Panche *et al.*, 2016; George *et al.*, 2014; Harbone and Williams 1992). The reported anti-inflammatory and anti-oxidant activity of the ethyl acetate fraction of *B. costatum* could be due to presence of flavonoids.

Among the five solvents (hexane, chloroform, ethyl acetate, n-butanol and water) used for fractionation, ethyl acetate was found to be more effective in extracting the maximum number of secondary metabolites. TLC profiling of hexane fraction only revealed the presence of triterpenes and steroids. Fractionation with chloroform did not yield any extract. Different solvent extracts of the phytochemicals provided further information about selecting appropriate solvent system for isolation of any compound from the plant extracts using chromatographic techniques (Birader and Rachetti 2013).

4. Conclusion

B. costatum stem bark contains important bioactive phytochemicals that support for its traditional use in the treatment of epilepsy, malaria, cancer and liver diseases. Isolation of these bioactive compounds can lead to development of novel drug for the treatment of various diseases that affect humanity.

5. Acknowledgement

The authors acknowledge the technical assistance of Kabiru Muhammad of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria.

6. References

Biradar, R.S. and Rachetti, D.B. 2013. Extraction of some secondary metabolites and thin layer chromatography from different parts of *Centella asiatica* L. *American Journal of Life Science*, 1(6): 243-247.

Eugene, T.Z.B., Oulaï, C. A., Fagbohoun , J. B., Gbocho, E. S.E. and Patrice, K. 2018. Polyphenols, flavonoids, carotenoids contents and mineral composition of *B. costatum* calyx: Their contribution to overall antioxidant. *International Journal of Food Science and Nutrition*, *3*(2): 38-43.

Gandhi, A.P., Joshi, K.C., Jha, K., Parihar, V.S., Srivastav, D.C., Raghunadh, P. and Tripathi, R.N. 2003. Studies on alternative solvents for the extraction of oil-I soybean. *International Journal of Food Science and Technology*, *38*(3): 369-375.

George, A., Ng, C.P., O'Callaghan, M., Jensen, G.S. and Wong, H.J. 2014. *In vitro* and *ex-vivo* cellular antioxidant protection and cognitive enhancing effects of an extract of *Polygonum minus* Huds (Lineminus) demonstrated in Barnes maze animal

model for memory and learning. *BMC Complementary and Alternative Medicine*, 14: 1-10.

Harborne, J.B and Williams, C.A. 2000. Advances in flavonoid research since 1992. *Phytochemistry*, 55(6): 481-504.

Laurence, L.B., Keith, L.P., Donald, K.B. and Lain, L.O.B. 2008. Goodman and Gilman's Manual of Pharmacology and Therapeutics. NewYork: McGraw-Hill Companies.

Leila, Z., Eliandra, D.S., Luisa, H.C., Anildo, C. J., Moacir, G.P., Bruno, S., Fatima, R. and Mena, B.S. 2007. Effect of crude extract and fractions from *Vitex megapotamica* leaves on hyperglycaemia in alloxan-diabetic rats. *Journal of Ethnopharmacology*. 109: 151-155.

Mohammed, N., Yaro, A.H. and Nazifi, A.B. 2018. *Bombax costatum* Pellegr. and Vuillet Stem Bark Extract Prevents Paracetamol and Carbon Tetrachloride-Induced Liver Injury in Rats. *Tropical Journal of Natural Product Research*, *2*(5): 220-226.

Mohammed, N., Anuka, J. A., Musa, A.M. and Yau, J. 2020. Anti-hepatofibrotic effect of ethyl acetate fraction of *Bombax costatum* Pellgr. EtVuillet stem bark against CCl_4-induced liver fibrosis in mice. *Journal of Medicinal Herbs*, 11(1): 1-7.

Mohammed, N.,Ahmadu, S., Jamilu, Yau., Bala, N. U., Rabiu, M. H., Najib, S. I., Khadija, U. and Aliyu, M. M. 2023. Hepatoprotective activity of ethyl acetate fraction of *bombax costatum* (pellegr. Et vuillet) stem bark against carbon tetrachlorideinduced acute liver injury in mice. *Archives of Pharmaceutical Sciences and Biotechnology Journal*. 3(2): 117-127.

Mohammed, N.,Ahmadu, S., Jamilu, Yau., Bala, N. U., Rabiu, M. H., Najib, S. I., Khadija, U. and Aliyu, M. M. 2023. Hepatoprotective activity of ethyl acetate fraction of *Bombax costatum* (pellegr. Et vuillet) stem bark against carbon tetrachlorideinduced acute liver injury in mice. *Archives of Pharmaceutical Sciences and Biotechnology Journal*, 3(2): 117-127.

Nazifi, A.B., Ahmed, A., Hassan, F.I., Mohammed, N., Danbala, A.A., Odoma, S. 2020. Comparative anticonvulsant activity of leaf, stem bark and root bark extracts of *Bombax costatum* Pellegr. Et Vuillet in acute models of epilepsy. *Tropical Journal of Natural Product Research*, *4*(10): 844-849.

Oyen, L.P.A. 2011. *Bombax costatum* Pellegr. & Vuillet. Record from PROTA4U. Wageningen, The Netherlands: PROTA.

Panche, A.N., Diwan, A.D. and Chandra, S.R. 2016. Flavonoids: an overview. *Journal of Nutritional Science*, 5(47): 1-15.

Randhawa, K., Kumar, D., Jamwal, A. and Kumar, S. 2015. Screening of antidepressant activity and *estimation* of quercetin from *Coccinia indica* using TLC densitometry. *Pharmaceutical Biology*, 53(12):1867-74.

Sonam, M., Singh, R.P. and Pooja, S. 2017. *Phytochemical* screening and TLC profiling of various extracts of *Reinwardtia Indica. International Journal of Pharmacognosy and Phytochemical Research*, 9(4):523-527.

Trease, G.E. and W.C. Evans, 1996. *A Text Book on Pharmacognosy*, (12^{th} Edn). Bailliere-Tindall. London: pp 153-475.

Wahab, O. M., Ayodele, A. E. and Moody, J. O. 2010. Thin layer chromatography phytochemical screening in some Nigerian Loranthaceae. *Journal of Pharmacognosy and Phytotherapy*, 2(5): 64-70.