

Full Length Research Paper

Antibacterial and phytochemical screening of crude ethanolic extracts of *Waltheria indica* Linn.

O. O. Olajuyigbe*, A. E. Babalola and A. J. Afolayan

Department of Botany, Phytomedicine Research Centre, University of Fort Hare, Alice, 5700, South Africa.

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The antibacterial activity and phytochemical properties of *Waltheria indica* Linn. was investigated, the result obtained showed that the ethanol extracts from the roots, stem and leaves were active against both Gram-positive and Gram-negative organisms. The qualitative phytochemical screening of these different parts indicated the presence of saponins, alkaloids, anthraquinones, flavonoids, tannins, phenols and cardiac glycosides at varied degrees. All the extracts showed antibacterial activities against both Gram-positive and Gram-negative bacteria. The highest antibacterial activity was obtained from the root extract, followed by the stem extract while the leaf extract was the least. The susceptibility of the different strains of Enterobacteriaceae to each extract varied with different parts investigated. This study indicated the potential efficacy of the *Waltheria indica* Linn. in the treatment of infections caused by the test organisms.

Key words: Phytochemical screening, enterobacteriaceae, phytochemicals, antibacterial activities, *Waltheria indica* extracts.

INTRODUCTION

In a constant attempt to improve the quality of life, men have used plants as source of food, shelter, clothing, medicine, cosmetics, and for seeking relief from hardship of life. Some plants are known as medicinal because they contain active substances that cause certain reactions, from relighting to the cure of diseases, on the humans (Silva Junior et al., 1994). Knowledge on medicinal plants sometimes means the only therapeutic resource of some communities and ethnic groups (Di Stasi, 1996).

Traditionally, plants are used as sources of treatment of diseases in different parts of the world (Eisenberg et al., 1993; Hostettmann et al., 2000) and their use contributes significantly to primary health care delivery (Holetz et al., 2002). They are regarded as invaluable sources of pharmaceutical products (Olalde Rangel, 2005). While traditional healers are still consulted in Nigeria as a first choice due to the fact that traditional medicine blends readily into the socio-cultural life of the people (Kela and Kufeji, 1995), healing powers are still

sought from plants by other countries of black Africa (Grierson and Afolayan, 1999; Anani et al., 2000). The screening of plant extracts and plant products for antimicrobial activity have shown that higher plants represent a potential source of novel antibiotic prototypes (Ateş and Erdoğan, 2003; Mthabe et al., 2006; Lategan et al., 2009).

Waltheria indica L. or sleepy morning, also known as velvet leaf, marsh-mallow, monkey bush, boater bush, leather coat, buff coat, and many other names (Burkill, 2000) belong to the family Sterculiaceae. It is found throughout the tropics and warmer subtropics. It apparently naturalized in Hawaii soon after the arrival of nonnative colonists (Wagner et al., 1990). The species is native to the New World where it occurs from Florida and Texas to Brazil. The plant has been used as an infusion or decoction where febrifugal, purgative, emollient, tonic, analgesic and astringent action is sought (Burkill, 2000). In the Turks and Caicos Islands, it is used to make herb tea. The plant produces a fiber that was formerly used for making cords, sacking, padding and sandals. Stems are used as a chew stick; extracts of the plant are used for treatment of cough and curing female sterility. In Hawaii, the root is chewed to relieve sore throat as well as

*Corresponding author. E-mail: funmijuyigbe12@yahoo.com.
Tel: +27730562219.

Table 1. Results of preliminary phytochemical screening of *W. indica*.

Plant parts	Alkaloids	Anthraquinones	Flavonoid	Tannin/Phenols	Saponin	Cardiac glycosides
Leaf	+	+++	+	+++	+++	+++
Root	+	+++	++	+++	+++	+++
Stem	+	+++	+	+	+++	+

+ = Trace; ++ = moderate, +++ = Abundant.

treatment of gonorrhoea and leprosy in humans. Stems are used as a chew stick while its extracts are used as an eye bath and a remedy for hemoptysis in Panama, treatment of cough and a cure for female sterility (Wagner et al., 1990).

From literature search, there is a lack of scientific proof to support the ethnomedicinal importance of this plant in the treatment of diarrhea, dysentery, pulmonary troubles and venereal diseases. Hence, this study was designed to investigate the qualitative phytochemical components of *W. indica* and determine the susceptibility of some bacteria to the plant extracts.

MATERIALS AND METHODS

Plant collection and identification

Fresh leaves, stems and roots of *W. indica* Linn. were collected from Babcock University, Ilishan Remo, Ogun State, Nigeria. The plant was authenticated by a Botanist at the Department of Botany and Microbiology, University of Ibadan, Ibadan.

Extract preparation

The root, stem and leaves of the plant were rinsed in clean water, air-dried at room temperature and pulverized using a milling machine. About 200 g of each pulverized sample were separately soaked in 500 ml of 95% ethanol in different 500 ml conical flasks for 48 h. The extractions were repeated for another two consecutive periods. The extracts were filtered with Whatman No.1 filter paper. The filtrates were evaporated to dryness under reduced pressure at a maximum temperature of 40°C using a rotary evaporator. Each dried crude ethanol extract was redissolved in ethanol to the required concentrations for the bioassay analysis. The corresponding concentration was expressed in term of mg of extract per ml of solvent (mg/ml).

Test organisms

A total number of 74 strains of pure cultures of different bacterial species were employed in this study. Pure cultures of different strains of *Citrobacter freundii* (4), *Escherichia coli* (5), *Enterococcus intermedius* (5), *Proteus morganella* (9), *Salmonella typhi* (15), *Enterococcus aerogenes* (5), *Klebsiella edwardskii* (10), *Proteus mirabilis* (4), *Proteus vulgaris* (4), *Staphylococcus aureus* (5) and *Streptococcus pyogenes* (8) were obtained from the Medical Microbiology Departments of three Teaching Hospitals in Southwestern Nigeria. Bacterial cultures were maintained on blood agar slant while antibacterial assay was carried out using Mueller Hinton agar.

Bacteriological analysis

In the hospital laboratory, the identity of each isolate was confirmed. The inoculum of each isolate was streaked out for discrete colonies with a wire loop following standard procedures (Cheesborough, 2006; Mordi and Erah, 2006). The culture plates were incubated at 37°C for 24 h and observed for pure colonies. The identity of all the bacteria were confirmed using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesborough (2006).

Bioassay

Antibacterial activity was determined by the well diffusion method according to the NCCLS (NCCLS, 1993). Petri plates containing 20 ml of Mueller Hinton agar were seeded with 100 µl portion of 24 h culture of the bacterial strains, swirled gently and allowed to solidify. Wells (6 mm diameter) were cut into the agar and 100 µl of each plant extracts were tested in a concentration of 40 mg/ml. Culture plates were incubated at 37°C for 24 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zones formed around the wells. A standard 5 µg ciprofloxacin disk was used as a positive control.

Preliminary phytochemical studies

The ethanolic extracts of leaves, stem and roots of *Waltheria indica* were analyzed by qualitative method for the presence of alkaloids, saponins, anthraquinones, flavonoids, phenol, and cardiac glycosides (Harborne, 2005).

RESULTS

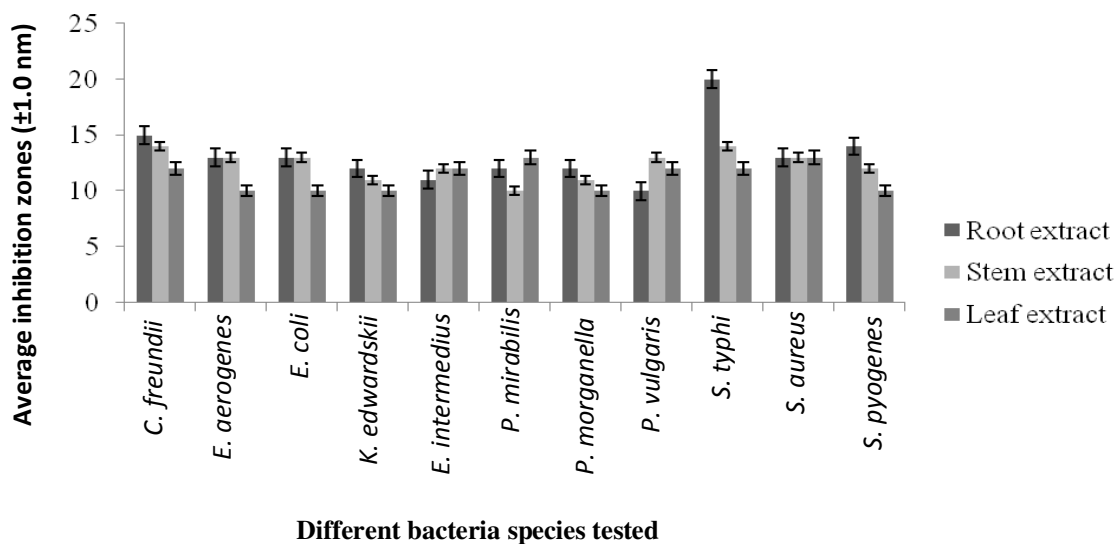
The qualitative phytochemical analysis of root, stem and leaf extracts of *W. indica* showed the presence of saponins, alkaloids, anthraquinones, flavonoids, tannins/phenols and cardiac glycosides at varied degrees (Table 1). High amount of saponins and anthraquinones were present in the three different parts of the plant than other phytochemicals. Tannins and cardiac glycosides were more observed in the roots and leaf extracts than in the stem extracts.

The different extracts of the *Waltheria indica* showed varied degrees of antibacterial activities and inhibited about 40 to 70% of the strains of each species of the test organisms. The average inhibition zones for these organisms ranged between 10 and 20 mm (Table 2). The susceptibility pattern of each bacterial species to the

Table 2. Average Inhibition zones of different bacterial species against ethanol extracts of *W. indica*.

Representative strain of each bacterial species used	Average inhibition zones (± 1.00 mm)				
	GR	RE	SE	LE	Cip
<i>Citrobacter freundii</i>	-	15	14	12	27
<i>Enterobacter aerogenes</i>	-	13	13	10	25
<i>Escherichia coli</i>	-	13	13	10	25
<i>Klebsiella edwardskii</i>	-	12	11	10	20
<i>Enterococcus intermedius</i>	-	11	12	12	20
<i>Proteus mirabilis</i>	-	12	10	13	21
<i>Proteus morganella</i>	-	12	11	10	20
<i>Proteus vulgaris</i>	-	10	13	12	18
<i>Salmonella typhi</i>	-	20	14	12	26
<i>Staphylococcus aureus</i>	+	13	13	13	27
<i>Streptococcus pyogenes</i>	+	14	12	10	19

GR = Gram's reaction; RE = Root extract; SE = Stem extract; LE = Leaf extract; Cip = Ciprofloxacin.

**Figure 1.** Susceptibility profiles of different bacterial species to extracts of *W. indica*.

root, leaf and stem extracts was indicated in Figure 1 while Figure 2 showed the number of susceptible strains of each species of the organisms tested. Although most of the isolates were susceptible to the different extracts, a high percentage of *Salmonella typhi* were more susceptible to the extracts from different parts of the plant. The root extract was the most active against all the isolates, followed by the stem extract while leaves extract was the least effective. Zones of inhibition ≥ 10 mm were considered as good antimicrobial activity. All the isolates were susceptible to 5 μ g of ciprofloxacin used as control. The average inhibition zones of these organisms from the extracts were less than those obtained from 5 μ g of the plant could be responsible for the observed ciprofloxacin. The phytochemical constituents detected in antibacterial

activity of *W. indica* though their exact mode of action remain unknown.

DISCUSSION

It is well known that infectious diseases account for high proportion of health problems, especially in the developing countries. There is an increasing trend in the emergence of resistance to antimicrobial agents which does not only result from poor quality drugs manufactured, patient non-compliance and irrational use of antimicrobial agents, but also due to spontaneous mutations within the microbial populations (Nester et al., 2002; Denyer et al., 2004). This situation has forced

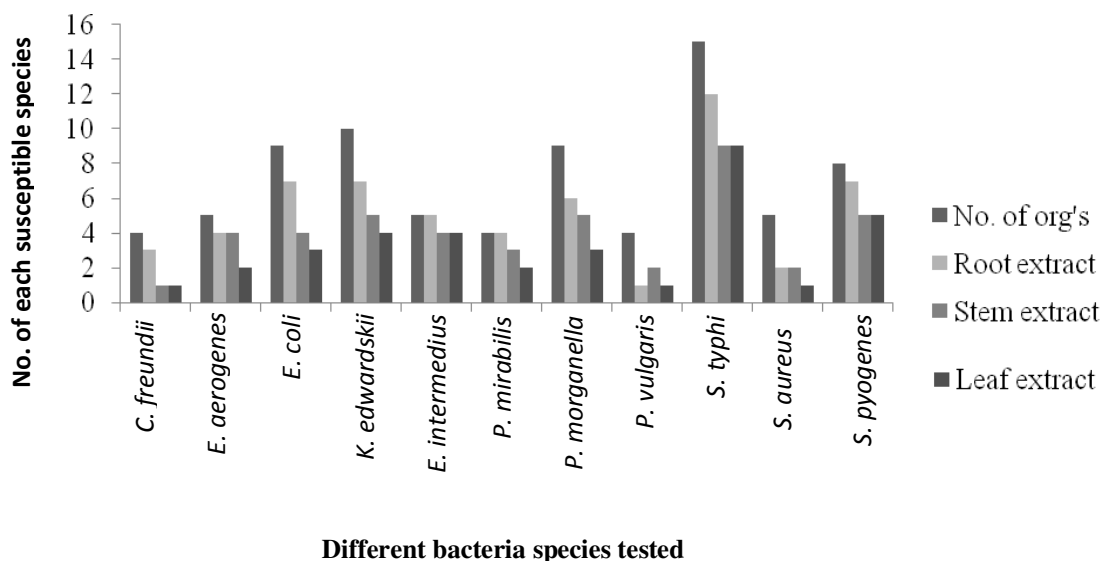


Figure 2. A comparison of the susceptibility of each bacterial species to each extract.

scientists to search for new antimicrobial substances from various sources such as medicinal plants. In the constant effort to improve the efficacy and ethics of modern medical practice, researchers are increasingly turning their attention to folk medicine as a source of new drugs.

In this study, the phytochemical screening of the root, stem and leaf extracts of *W. indica* revealed the presence of alkaloids, anthraquinones, cardiac glycosides, phenols, tannins and saponins. These compounds are known to have antibacterial activity against pathogens and could be used traditionally for therapeutic purposes (Usman and Osuji, 2007).

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids which have been found *in vitro* to have antimicrobial properties (Cowan, 1999; Dahanukar et al., 2000). The activities of all the extracts against both Gram-negative and Gram-positive bacteria agreed with previous studies which indicated that plant extracts are capable of inhibiting these two groups of bacteria (Jimoh et al., 2008; Rahman et al., 2009). These antibacterial activities against both Gram-positive and Gram-negative bacteria may be indicative to the presence of broad spectrum antibiotic compounds or general metabolic toxins in addition to the pharmacologically active metabolites (Xu et al., 2000; Kostova and Dinchev, 2005) in *W. indica*.

This result may be explained to the fact that phenolic compounds and their derivatives are considered as antiseptic agents changing the cell protein nature (denaturation) and increasing the permeability of cell membranes (Farag et al., 1989; Feeny et al., 1998) in these bacteria. Phillipson and O'Neill (1987), also,

indicated that the mechanism of action of aromatic planar quaternary alkaloids in the extracts could be attributed to their ability to interchelate with DNA. Flavonoids activity may be explained to be a result of their complex ability with extracellular and soluble proteins as well as bacterial cell walls (Cowan, 1999) while more lipophilic flavonoids may disrupt microbial membranes (Tsuchiya et al., 1996). While the antimicrobial actions of tannins have been associated with their ability to couple with polysaccharides (Ya et al., 1988), they also inactivate microbial adhesions, enzymes, cell envelope and precipitate microbial protein (Scalbert, 1991).

Though Kolapo et al. (2009) reported that the stem barks of medicinal plants generally show high antimicrobial activity than the leaves and many other reports showed that plants leaves possess high antimicrobial activity than other parts (Alves et al., 2000; Ndukwe et al., 2007; Nwaogu et al., 2008), our study showed that the root extract of this plant was more active than the stem extracts while the leaf extracts indicated the least activities against bacterial pathogens.

Conclusions

The susceptibility of the various bacteria employed in this study indicated that *W. indica* is a significant plant that will be useful in the treatment of enteric diseases in which these bacteria are associated. Therapeutically, infections caused by *Salmonella typhi* would be more relieved with the use of *W. indica*. The use of roots of *W. indica* in decoction would likely yield better antibacterial effects than the other plant's part. While *W. indica* could be a source of new antibiotic compounds, this study justified

the relevance of this plant in the treatment of microbial infections by the rural communities.

REFERENCES

- Alves TM, Silva AF, Brandao M, Grandi TS, Samania EF, Junior AS, Zani CL (2000). Biological screening of Brazilian medicinal plants. *Rio de Janeiro*, 95(3): 367-373.
- Anani K, Hudson JB, de Souza C, Akpagana K, Towers GHN, Arnason JT, Gbeassor M (2000). Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. *Pharm. Biol.*, 38(1): 40-45.
- Ateş DA, Erdoğan ÖT (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turk. J. Biol.*, 27: 157-162.
- Burkill HM (2000). *The useful plants of West Tropical Africa*. Royal Botanic Gardens, Kew, UK, p. 686.
- Cheesborough M (2006). *Medical Laboratory Manual for Tropical Countries, II Microbiology (ELBS)*, Butterworth, Kent, U.K, pp. 23-78.
- Cowan SJ, Steel KJ (1974). *Cowan and Steel manual for identification of medical bacteria*, 2nd edn Cambridge University Press, London, pp. 176-232.
- Cowan MM (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12: 564-582.
- Dahanukar SA, Kulkarni RA, Rege NN (2000). Pharmacology of medicinal plants and natural products. *Ind. J. Pharmacol.*, 32: S81-S118.
- Denyer SP, Hodges NA, Gorman SP (2004). Bacterial resistance to antibiotics and clinical uses of antimicrobial drugs. In Hugo & Russell's *Pharmaceutical Microbiology*. 5th Ed. Blackwell Publishing, Chapt., (13-14): 220-250.
- Di Stasi LC (1996). *Arte, ciência e magia*. In LC Di Stasi, CA Hiruma-Lima (eds), *Plantas Mediciniais: Arte e Ciência, São Paulo*, pp. 15-21.
- Eisenberge DM, Kesler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL (1993). *Unconventional Medicine in the United States: Prevalence, costs and Patterns of use*. *N. Engl. J. Med.*, 328: 246-252.
- Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA (1989). Antibacterial activity of some Egyptian spices essential oils. *J. Food Prot.*, 52: 665-667.
- Feeny P (1998). Inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin. *J. Phytochem.*, 8: 2119-2126.
- Grierson DS, Afolayan AJ (1999). An Ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. *J. Ethnopharmacol.*, 67: 327-332.
- Harborne JB (2005). *Phytochemical methods – A guide to modern techniques of plant analysis*. 3rd edition. New Delhi: Springer Pvt. Ltd.
- Holez FB, Pessini GL, Sanches NR, Cortez DAG, Nakamura CV, Dias Filho BP (2002). Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. *Mem. Inst. Oswaldo Cruz.*, 97: 1027-1031.
- Hostettmann K, Marston A, Ndojoko K, Wolfender J (2000). The potential of Africa Plants as a source of drug. In: *Curr. Organic Chem.*, 4: 973-1010.
- Jimoh FO, Adedapo AA, Aliero AA, Afolayan AJ (2008). Polyphenolic contents and biological activities of *Rumex ecklonianus*. *Pharm. Biol.*, 46(5): 333-340.
- Kela SL, Kuféji JH (1995). Screening of some Nigerian plants for bactericidal activity. *J. Microbiol.*, 10: 18-22.
- Kostova I, Dinchev D. (2005). Saponins in *Tribulus terrestris*—chemistry and bioactivity. *Phytochem Rev.* 4(2-3): 111–137.
- Kolapo AL, Okunade MB, Adejumbi JA, Ogundiya MO (2009). Phytochemical composition and antimicrobial activity of *Prosopis africana* against some selected oral pathogens. *World J. Agric. Sci.*, 5: 90-93.
- Lategan CA, Campbell WE, Seaman T, Smith PJ (2009). The bioactivity of novel furanoterpenoids isolated from *Siphonochilus aethiopicus*. *J. Ethnopharmacol.*, 121: 92-97.
- Mthabe MC, Niikolova RV, Lall N, Nyazema NZ (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *J. Ethnopharmacol.*, 105: 286-293.
- Mordi RM, Erah PO (2006). Susceptibility of common urinary tract isolates to the commonly used antibiotics in a tertiary hospital in Southern Nigeria. *Afr. J. Biotechnol.*, 5: 1067-1071.
- NCCLS (National Committee for Clinical Laboratory Standards) (1993). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard, M7-A3*. NCCLS, Villanova, PA.
- Ndukwe IG, Bello AI, Habila JD, John C (2007). Phytochemical and antimicrobial screening of the crude petroleum spirit and methanol extracts of the stem bark, leaves and roots of *Ficus thoningii* (blume). *Afr. J. Biotech.*, 6: 2645-2649.
- Nester MT, Anderson DG, Roberts Jr CE, Pearsall NN, Nester MT (2002). *Microbiology - A human perspective*. Genitourinary Infections and antimicrobial medications. 3rd Ed. McGraw Hill. Madrid., 21-25: 495-664.
- Nwaogu LA, Alisi CS, Igwe CU, Ujowundu CO (2008). A comparative study of the antimicrobial properties of the ethanolic extracts of *Landolphia owariensis* leaf and root. *Afr. J. Biotech.*, 7: 368-372.
- Olalde Rangel JA (2005). The systemic theory of living systems and relevance to CAM. Part I: The theory. *Evid Based Comp. Alt. Med.*, 2: 13–18.
- Phillipson JD, O'Neill MJ (1987). New leads to the treatment of protozoal infections based on natural product molecules. *Acta. Pharm. Nord.*, 1: 131-144.
- Rahman MM, Sheikh MMI, Sharmin SA, Islam MS, Rahman MA, Rahman MM, Alam MF (2009). Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. *CMU. J. Nat. Sci.*, 8(2): 219.
- Scalbert A (1991). Antimicrobial properties of tannins. *Photochem.*, 30: 3875-3883.
- Silva Junior AA, Vizotto VJ, Giongi E, Macedo SG, Marques LF (1994). *Plantas medicinais, caracterização e cultivo*. EPAGRI. Bol. Técnico Florianópolis, pp. 68: 1-71.
- Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M (1996). Comparative study on the antibacterial activity of phytochemical flavonones against methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol.*, 50: 27-34.
- Usman H, Osuji JC (2007). Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia leavis*. *Afr. J. Trad. CAM*, 4(4): 476-480.
- Wagner WL, Herbst DR, Sohmer SH (1990). *Manual of the flowering plants of Hawai'i*. vols., Bishop Museum Special Publication 83. Honolulu: University of Hawaii Press and Bishop Museum Press, p. 1280.
- Xu YX, Chen HS, Liang HQ, Gu ZB, Lui WY, Leung WN, Li TJ (2000). Three new saponins from *Tribulus terrestris*. *Planta Med.* 66(6): 545–550.
- Ya C, Gaffney SH, Lilley TH, Haslam E (1988). Carbohydrate - "polyphenol complexation. p. In: Hemingway RW and Karchesy JJ. (ed.). *Chemistry and significance of condensed tannins*. Plenum Press, New York.