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Comparative analysis of aqueous and methanolic *Raphanus sativus* root extracts: Composition, yield and pathogen-specific bioactivity

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ABSTRACT

Raphanus sativus root extracts exhibited solvent dependent distributions of phytochemicals and distinct antimicrobial profiles: both qualitative and quantitative analyses indicated that aqueous and methanolic extracts contained alkaloids, flavonoids, carbohydrates, and proteins, whereas methanol uniquely produced saponins and terpenoids, and water uniquely yielded tannins; flavonoids were most prevalent in water (4.0 mg/g compared to 3.0 mg/g in methanol), while alkaloids were more abundant in methanol (3.2 mg/g versus 2.0 mg/g), underscoring the differences in solubility driven by polarity. Antibacterial tests demonstrated dose-dependent inhibition, with the methanolic extract generally exhibiting greater potency, particularly against *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Bacillus cereus,* likely due to the membrane-disrupting effects of saponins and terpenoids, while the aqueous extract showed significant activity against *Staphylococcus aureus*. Antifungal outcomes were specific to both strain and dose: the aqueous extract effectively *inhibited Aspergillus flavus* and *Penicillium* sp., while methanol achieved a remarkable peak inhibition of *Fusarium* sp. at an intermediate dose (50 µL), but showed reduced efficacy at higher volumes, indicating potential narrow therapeutic windows or antagonistic interactions. These results suggest that the choice of solvent plays a crucial role in determining extract composition and bioactivity, and they advocate for further isolation of active compounds and the assessment of minimum inhibitory concentrations.

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INTRODUCTION

Medicinal plants have historically been a fundamental aspect of traditional medicine and continue to serve as important sources for new antimicrobial agents, particularly in light of the rising prevalence of drug-resistant pathogens. The quest for phytoconstituents derived from plants with antimicrobial properties has become increasingly significant due to the limitations of conventional antibiotics and the demand for safer, more accessible alternatives (Janjua and Shahid, 2013).

Raphanus sativus L., commonly referred as radish, belongs to the Brassicaceae family and is extensively cultivated globally as an edible root vegetable. Although primarily utilized as a food source, radish has been recognized in traditional medicinal practices for its various pharmacological effects, which include antimicrobial, antioxidant, and anti-inflammatory properties. Notably, the root is acknowledged as the most bioactive component of the plant, demonstrating greater antibacterial efficacy than extracts from the stem and leaves (Kaymak *et al.,* 2015).

Phytochemical screening is an essential analytical method for evaluating the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and steroids, all of which contribute to the medicinal characteristics of plant extracts (Beevi *et al.,* 2009). Prior research on the root of *R. sativus* has identified these significant phytoconstituents, including flavonoids (rutin, quercetin, and narigenin), isothiocyanates (AITC, PITC, BITC), alkaloids, and phenolic compounds, underscoring its potential for medicinal use.

The in vitro antimicrobial efficacy of *R. sativus* root extracts has been established against both Gram-positive bacteria and Gram-negative bacteria through the application of agar well diffusion and agar diffusion techniques (Shukla *et al.,* 2011). The antibacterial effectiveness has been found to be on par with standard antibiotics such as gentamycin and tetracycline, with minimum inhibitory concentration (MIC) values ranging from 20 to 40 mg/mL. Importantly, the root extract exhibited significant antioxidant capacity.

Although previous studies have reported the phytochemical composition and antimicrobial characteristics of *R. sativus* root peel extracts, seed oil, and various solvent fractions, there is still a pressing need for thorough investigations that specifically examine the whole root extract utilizing different solvents to enhance extraction efficiency and antimicrobial effectiveness (Singh and Singh, 2013). This research intends to perform phytochemical analysis and assess the in vitro antimicrobial activity of *Raphanus sativus* L. root extract, thus contributing to the scientific substantiation of its traditional medicinal applications and investigating its potential as a source of natural antimicrobial compounds.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh roots of *Raphanus sativus* L. were collected from the experiment pot culture, Thanjavur, Tamil Nadu, India and authenticated by a botanist; a voucher specimen was deposited in the institutional herbarium. The roots were washed thoroughly with distilled water, peeled, and the peels were air-dried at room temperature (25–30°C) until constant weight. Dried peels were finely powdered using an electric grinder and stored in sterile, airtight containers at room temperature until extraction.

For crude extraction, a known amount of root peel powder was macerated with distilled water and methanol (1:10 w/v) in conical flasks for 24–48 h at room temperature with occasional shaking. The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated to dryness on a water bath or rotary evaporator. Dried extracts were weighed, transferred to sterile vials, and stored at 4°C. For bioassays, extracts were re-dissolved in distilled water or suitable solvent to obtain a 20% w/v stock, and diluted to the desired test volumes (25, 50, 75 and 100 µL per well).

Qualitative phytochemical screening

Aqueous and methanolic extracts of *R. sativus* root peels were screened for major secondary metabolites (alkaloids, coumarins, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins and terpenoids) using standard phytochemical procedures (Harbone, 1973).

Alkaloids were detected by Mayer's test after acidification with HCl and treatment with Mayer's reagent; formation of a greenish/cream precipitate indicated a positive result.

Carbohydrates were identified by Molisch's test, showing a reddish-brown ring at the interface after addition of Molisch's reagent and concentrated H_2SO_4. Flavonoids were detected by the alkaline reagent test (yellow color with NaOH, discharged on acidification), and anthraquinone glycosides by Borntrager's test after acid hydrolysis, chloroform extraction, and treatment with dilute ammonia. Phenols were tested using ferric chloride (violet/blue color), proteins by a biuret-type reaction using NaOH and $CuSO_4$ (violet color), and saponins by foam/emulsion formation upon vigorous shaking with water and oil. Steroids were evaluated using the Liebermann–Burchard reaction (green upper layer), tannins by $FeCl_3$ (greenish-black precipitate), and terpenoids by development of grey color after sulfuric acid treatment of the chloroform extract.

Quantitative phytochemical estimation

All quantitative determinations were performed in triplicate and expressed relative to the dry weight of plant material.

Total alkaloids (Harbone, 1973) were estimated by extracting 1 g of powder with 10% acetic acid in ethanol, concentrating the filtrate, precipitating with concentrated NH_4OH, and determining the dry weight of the precipitate gravimetrically.

Reducing carbohydrates were quantified using Fehling's method (Trease and Evans, 1983), with freshly prepared Fehling's A and B, heating the extract with reagent, and expressing reducing sugars from titration or gravimetric data using a standard curve.

Total flavonoids (Boham and Kocipai-Abyazan, 1994) were measured gravimetrically by repeated extraction of 1 g of sample with 80% methanol, evaporation to dryness, and expressing the residue as % flavonoids.

Cardiac glycosides were determined from (Harbone, 1973) ethanolic extracts purified with lead acetate and Na_2HPO_4 and reacted with Buljet's reagent; absorbance was read spectrophotometrically against a reagent blank and calculated from a calibration curve.

Total proteins were quantified using the Bradford assay (Harbone, 1973) 100 µL of extract was mixed with Bradford reagent, incubated for 5 min, and absorbance was measured at 595 nm using bovine serum albumin (BSA) standards.

Saponins were (Obodoni and Ochuko, 2001) estimated by successive extraction of 20 g of powder with 20% ethanol, partitioning with diethyl ether and n-butanol, washing with 5% NaCl, evaporating to dryness, and expressing the residue gravimetrically as % saponins.

Tannins were measured (Van-Burden and Robinson, 1981) colorimetrically after methanol aqueous extraction using $FeCl_3$ and potassium ferrocyanide, and absorbance at a suitable visible wavelength was converted to tannin content using tannic acid standards.

Total terpenoids were obtained by partitioning the methanolic extract with petroleum ether, evaporating the petroleum ether fraction to dryness in pre-weighed vials, and calculating terpenoid yield gravimetrically as % of the initial extract weight (Harbone, 1973).

***In vitro* antimicrobial activity**

Test microorganisms and culture media

The antimicrobial activity (Daoud *et al.,* 2015) of the root peel extracts was evaluated against bacterial

strains (*Bacillus* sp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and fungal strains (*Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium* sp., *Penicillium* sp.). Bacteria were cultured on Nutrient Agar and fungi on Potato Dextrose Agar (PDA). Standardized inoculate were prepared from overnight cultures or spore suspensions adjusted to appropriate densities.

Agar well diffusion assay

Antimicrobial activity was assessed by agar well diffusion. Standardized microbial suspensions (1 mL) were mixed with molten Nutrient Agar (bacteria) and PDA (fungi) at 45–50°C and poured into sterile Petri dishes. After solidification, wells of 6 mm diameter were punched aseptically with a sterile cork borer. Each well was filled with 25, 50, 75 or 100 µL of extract (20% w/v) using a micropipette; solvent controls and standard antimicrobial agents were included as negative and positive controls, respectively. Plates were kept at 4°C for 30 min to allow pre-diffusion and then incubated at 37°C for 18–24 h for bacteria and 25–30°C for 48–72 h for fungi. Zones of inhibition around the wells were measured in millimetres (including well diameter), and all experiments were performed in triplicate.

RESULTS AND DISCUSSION

Phytochemical screening and quantitative estimation

The qualitative screening (Table 1) and subsequent quantitative verification (Fig. 1) of *Raphanus sativus* root extracts revealed a diverse profile of secondary metabolites, heavily influenced by the polarity of the extraction solvents used Water, being a polar and a green solvent, is particularly effective for extracting polar compounds such as phenolics, flavonoids, terpenoids, and various nitrogen-containing substances (Dhanani *et al.,* 2017).

Qualitative analysis demonstrated that both aqueous and methanol extracts successfully recovered core compounds, including alkaloids, carbohydrates, flavonoids, and proteins. The results indicated the presence of polar compounds in polar solvents in comparison to the non-polar solvents, similar to our results Anyanele *et al.* (2022). However, distinct variations emerged among specific metabolite classes. Saponins and terpenoids were exclusively detected in the methanol extract, while tannins were selectively isolated in the aqueous phase.

Table 1. The qualitative analysis of phytochemical compounds of *Raphanus sativus* root extract

Phytochemical compounds	Different solvents	
	Aqueous	Methanol
Alkaloids	+	+
Carbohytrade	+	+
Coumarins	-	-
Flavonoids	+	+
Glycosides	-	-
Phenols	-	-
Protein	+	+
Saponins	-	+
Steroids	-	-
Tannin	+	-
Terpenoids	-	+

(+) present (-) absent

Conversely, coumarins, glycosides, phenols, and steroids were entirely absent in both extracts under the tested conditions. The aqueous extract contained tannins, saponins, cardiac glycosides, alkaloids and flavonoids whereas methanol extract of the same species was found to contain terpenoids, cardiac glycosides, steroids, flavonoids and alkaloids which are similar to our results (Devkota and Sahu, 2019).

Quantitative evaluation corroborated these findings, providing insight into total yield variations. Flavonoids represented the most abundant phytochemical class in the aqueous extract, yielding a peak concentration of 4.0 mg/g, compared to 3.0 mg/g in the methanol extract. This indicates that the highly polar aquatic environment serves as an optimal matrix for flavonoid extraction in *R. sativus* (Kong *et al.*, 2024).

Alkaloids, on the other hand, showed higher affinity for the organic solvent, yielding 3.2 mg/g in methanol compared to 2.0 mg/g in water. Carbohydrates showed slightly higher accumulation in the aqueous phase (3.0 mg/g vs. 2.4 mg/g), while proteins

remained identical across both solvents at 3.0 mg/g. Among the solvent-specific compounds, methanol yielded 2.0 mg/g of saponins and 1.0 mg/g of terpenoids, while the aqueous extract yielded 2.0 mg/g of tannins. Phenolic compounds decrease oxidative stress brought on by heavy metals by working as metal chelators and antioxidants (Ahmed *et al.*, 2010).

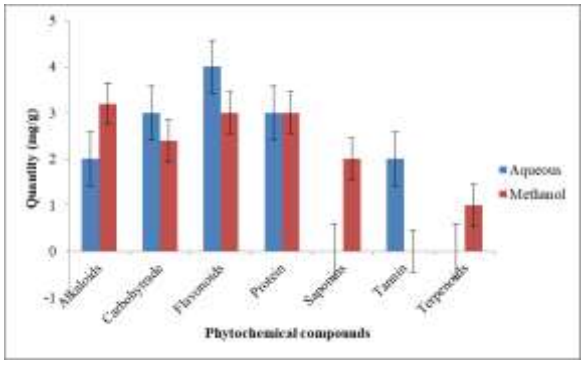


Fig. 1. The quantitative analysis of phytochemical compounds of *Raphanus sativus* root extract

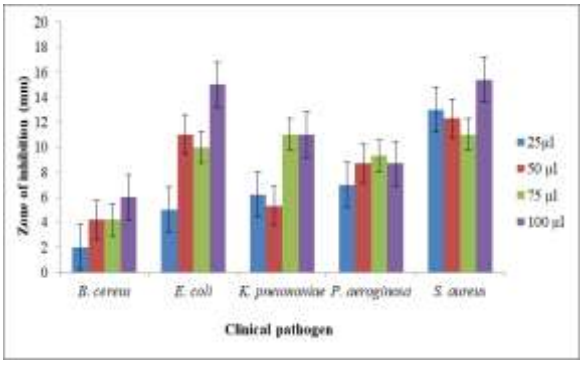


Fig. 2. The antibacterial activity of *Raphanus sativus* root with aqueous extract against bacteria

The variation in these values highlights how solvent selection governs the solubility of specific bioactive compounds. The strong presence of flavonoids and alkaloids is particularly significant, as these compounds are widely documented to mediate antimicrobial pathways via cellular membrane disruption and enzyme inhibition. Flavanoids also help prevent plants from metal-induced oxidative damage by scavenging reactive oxygen species (Khan *et al.*, 2018).

Antimicrobial potency against clinical pathogens

Antibacterial profile

The evaluation of *R. sativus* root extracts against clinical bacterial pathogens displayed clear, dose-dependent inhibitory patterns, with the methanol extract consistently outperforming its aqueous counterpart across almost all tested strains (Fig. 2 and 3).

The aqueous extract exhibited modest to moderate antibacterial properties (Fig 2). *Staphylococcus aureus* was the most susceptible strain, showing zones of inhibition (ZOI) ranging from 13.0 mm at 25 µL to a maximum of 15.4 mm at 100 µL. In contrast, *Bacillus cereus* demonstrated minimal susceptibility, yielding a maximum ZOI of only 6.0 mm at the highest concentration. *Escherichia coli* and *Kleinfelsia pneumoniae* displayed intermediate sensitivity, peaking at 15.0 mm and 11.0 mm at 100 µL, respectively.

The methanol extract showed broader and more potent efficacy (Fig. 3). While it was less effective against *S. aureus* at the lowest dose (5.3 mm at 25 µL), it reached parity with the aqueous extract at higher doses, capping at 13.2 mm. Notably, it demonstrated drastically improved performance against *K. pneumoniae* (reaching 14.4 mm) and *P. aeruginosa* (peaking at 15.0 mm at the 50 µL threshold before tapering). Furthermore, its effect on *B. cereus* was substantially enhanced compared to the water extract, scaling up to 11.2 mm at 100 µL.

This enhanced efficacy of the organic extract is highly correlated with the presence of saponins and terpenoids alongside alkaloids. Saponins are known to disrupt bacterial cell membrane permeability, which likely explains why the organic fraction managed to breach the formidable outer membranes of Gram-negative pathogens like *P. aeruginosa* and *K. pneumoniae* more effectively than the water fraction. These compounds can control the surrounding microbiome by suppressing harmful microorganisms while promoting the growth of beneficial microbial communities that support

seedling development (Abouzeid *et al.*, 2023; Laouane *et al.*, 2024).

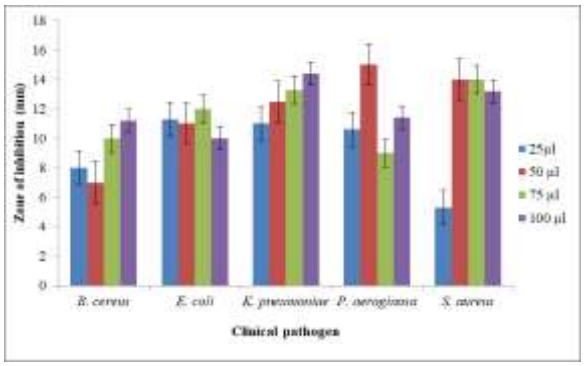


Fig. 3. The antibacterial activity of *Raphanus sativus* root with methanol extract against bacteria

Antifungal profile

The antifungal bioassays (Fig. 4 and 5) revealed complex, non-linear dynamics, particularly highlighting specific vulnerabilities among the clinical fungal strains.

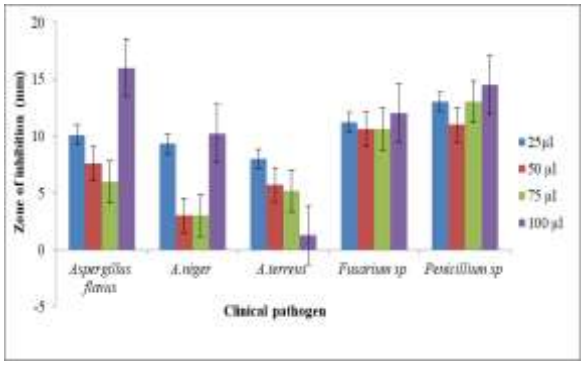


Fig. 4. The antifungal activity of *Raphanus sativus* rhizome with aqueous extract against fungi

The aqueous extract demonstrated strong (Fig. 4), targeted activity against *Aspergillus flavus*, with the ZOI expanding dramatically from 10.0 mm (25 µL) to 16.0 mm (100 µL). *Penicillium* sp. also showed uniform susceptibility, peaking steadily at 14.5 mm. Conversely, *Aspergillus terreus* displayed a negative dose-response curve, where increasing concentrations led to a sharp contraction of the zone of inhibition, falling from 8.0 mm down to a negligible 1.3 mm at 100 µL. This indicates a potential hormetic effect or fungal adaptation at higher aqueous doses.

The methanol extract produced a pronounced, anomalous inhibitory peak against *Fusarium* sp. at 50 µL (Fig. 5), yielding a ZOI of 23.0 mm, the largest zone observed in the study. This effect dropped significantly to around 11.0 mm at higher doses, suggesting a narrow, optimal therapeutic window or cumulative compound interactions at elevated volumes (Salvosa and Cubillan, 2004). *A. niger* and *A. terreus* showed steady dose-dependent responses to the methanol extract, with *A. niger* reaching 14.0 mm and *A. terreus* reaching 10.0 mm at 100 µL.

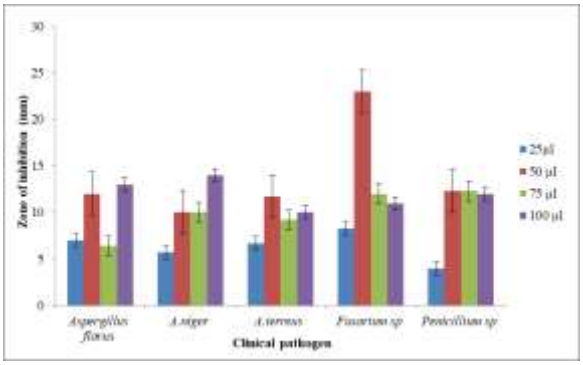


Fig. 5. The antifungal activity of *Raphanus sativus* rhizome with methanol extract against fungi

CONCLUSION

Taken together, the data indicates that *Raphanus sativus* roots contain highly potent, polar-extractable antimicrobial agents. While the aqueous extract acts as an efficient medium for retrieving high-yield flavonoids with specialized activity against *S. aureus* and *A. flavus*, the methanol extract succeeds in unlocking a broader multi-metabolite shield (alkaloids, saponins, and terpenoids). This makes the organic extract highly proficient at subduing stubborn clinical pathogens like *Pseudomonas aeruginosa* and *Fusarium* sp. These findings support the traditional therapeutic claims of radish roots and underscore their potential as scaffolds for developing botanical preservation or natural antibiotic agents.

REFERENCES

Abouzeid S, Beutling U, Elekhnawy E, Dirk S. 2023. Antibacterial and antibiofilm effects of allelopathic compounds identified in *Medicago sativa* L. seedling exudate against *Escherichia coli*. Molecules **28**, 2645.

Ahmed S, Khan MA, Iqbal Z. 2010. Phytochemical analysis and biological activities of medicinal plants. Journal of Medicinal Plants Research **4(12)**, 1125–1132.

Anyanele WC, Anyanele SI, Ejidike CS. 2022. Comparative study on the phytochemical analysis of the leaves and stem of *Ageratum houstonianum* Mill. Asian Plant Research Journal, 49–55.

Beevi SS, Mangamoori LN, Dhand V, Ramakrishna DS. 2009. Isothiocyanate profile and selective antibacterial activity of root, stem, and leaf extracts derived from *Raphanus sativus* L. Foodborne Pathogens and Disease **6(1)**, 129–136.

Boham BA, Kocipai-Abyazan R. 1994. Flavonoids and condensed tannins from leaves of *Vaccinium vaticulatum* and *V. calycinium*. Pacific Science **48**, 458–463.

Daoud A, Malika D, Bakari S, Hfaiedh N, Mnafgui K, Kadri A. 2015. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two Tunisian cultivars. Arab. J. Chem. (in press).

Devkota A, Sahu A. 2019. Evaluation of *Ageratum houstonianum* Mill. leaves extracts against phytopathogenic fungi. Indian Journal of Natural Products and Resources **10(3)**, 181–187.

Dhanani T, Shah S, Gajbhiye NA, Kumar S. 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. Arabian Journal of Chemistry **10**, 1–7.

Harbone JB. 1973. Phytochemical methods. Chapman and Hall, Ltd., London, pp. 49–188.

Janjua S, Shahid M. 2013. Phytochemical analysis and in vitro antibacterial activity of root peel extract of *Raphanus sativus* L. var. *niger*. Advancement in Medicinal Plant Research **1(1)**, 1–7.

Kaymak HC, Ozturk S, Ercisli S, Guvenc I. 2015. In vitro antibacterial activities of black and white radishes (*Raphanus sativus* L.). Comptes Rend. L Acad. Bulgare Des. Sci. **68**, 201–208.

Khan RA, Khan MR, Sahreen S. 2018. Protective effects of flavonoids against heavy metal-induced toxicity: A review. Environmental Science and Pollution Research **25(3)**, 2155–2168.

Kong C, Li Z, Li F, Xia X, Wang P. 2024. Chemically mediated plant–plant interactions: Allelopathy. Plants **13**, 626.

Laouane H, Bouajaj S, Darrag ELM, Benyamna A, Hamrouni L, Romane A. 2024. Assessment of the allelopathic effect, antimicrobial potential and chemical composition of *Senecio anteuphorbium* L. essential oil. Records of Natural Products **18(4)**, 436–445.

Obodoni BO, Ochuko PO. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure Applied Science **8(3)**, 203–208.

Salvosa FAM, Cubillan LP. 2004. *In vitro* evaluation of natamycin 5% suspension against *Aspergillus flavus*, *Fusarium solani*, and *Candida parasilopsis*. Philippine Journal of Ophthalmology **29(1)**, 26–28.

Shukla S, Chatterji S, Yadav DK, Watal G. 2011. Antimicrobial efficacy of *Raphanus sativus* root juice. Int. J. Pharm. Pharm. Sci. **3(5)**, 89–92.

Singh P, Singh J. 2013. Medicinal and therapeutic utilities of *Raphanus sativus*. Int. J. Plant Anim. Environ. Sci. **3**, 103–105.

Trease GE, Evans WC. Pharmacognosy. 12th ed. Balliere Tindall, Eastbourne, UK.

Van-Burden TP, Robinson WC. 1981. Formation of complexes between protein and tannic acid. Journal of Agricultural Food Chemistry **1**, 77.