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Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2024.v16.i01.pp107-116

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF MORINGA OLEIFERA LEAF EXTRACTS

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Article info

Received date:20-09-2023
Accepted date:11-12-2023
Published date:12-04-2024
ISSN;0975-8216

Keywords:

Moringa oleifera, Phytochemical screening, Antioxidant activity, Leaf extracts, Polyphenols

Abstract

Moringa oleifera leaves are widely eaten and used in traditional medicine, and a large body of laboratory work describes their phytochemistry and antioxidant behaviour. This review summarises published reports on how leaf extracts are prepared, how qualitative screening and colorimetric quantification are carried out, and which antioxidant assays are most often applied. It also discusses what the literature says about the compounds responsible, the influence of solvent and plant origin, and the limits of in vitro assays. Extracts rich in phenolics and flavonoids generally show strong radical scavenging, but methods differ so much that comparison is difficult. The review reports no new experimental data.

1. INTRODUCTION

Moringa oleifera Lam., known as the drumstick or horseradish tree, is native to the sub-Himalayan regions of India and is now grown across tropical and subtropical Asia, Africa and Latin America. Leaves, pods, seeds, roots and flowers all have food or medicinal uses [1, 2]. The leaves attract the most scientific attention because they are eaten as a vegetable, dried as a powder and promoted as a nutritional supplement, particularly where diets are low in vitamins and protein [3, 4]. A large literature has grown around the claim that the leaves are also a rich source of antioxidants, and the topic is a standard subject for pharmacognosy teaching laboratories as well as for more ambitious research programmes.

Antioxidant activity matters in pharmaceutical sciences because reactive oxygen species are implicated in inflammation, cardiovascular disease, diabetes and ageing [5, 6]. Plants contain a variety of molecules that can donate electrons or hydrogen atoms to unstable radicals, and phenolic compounds are the most discussed [7, 8]. Whether a plant extract that performs well in a test tube will have any benefit after ingestion is a separate and much harder question, one that the literature on polyphenol bioavailability treats with caution [9, 10]. This review therefore tries to keep what has been measured distinct from what has been inferred.

The review is organised around the way a typical study proceeds. It begins with the botanical and chemical background, then describes extraction and qualitative screening, colorimetric quantification of phenolics and flavonoids, and the antioxidant assays most commonly applied. Findings reported by other groups on Moringa leaf extracts are then compared in table 1 and discussed, followed by safety considerations and the limits of the evidence. It draws on published reviews and experimental papers and reports no new data of its own. Where the literature disagrees, the disagreement is described and not smoothed over.

Table 1: Representative published findings on the phytochemistry and antioxidant activity of Moringa oleifera leaf extracts

Topic	Reported finding	Ref.
Flavonoids quercetin and kaempferol glycosides	Prominent in leaf extracts by liquid chromatography; linked to anti-inflammatory effects in cell models	[11, 12]
Glucosinolates glucomoringin and breakdown products	Distinctive to the genus; isothiocyanates proposed as additional bioactive agents	[13, 14]
Phenolic acids and chlorogenic acids	Reported across leaf extracts and contribute to reducing capacity	[11, 15]
Solvent choice	Aqueous alcohols often extract more phenolics and give stronger activity than pure solvents	[16, 17]
Plant origin and maturity	Phenolic content and activity differ between agroclimatic origins and leaf stages	[16, 18]
Folin-Ciocalteu assay	Reducing substances such as ascorbic acid inflate phenolic values	[19, 20]
DPPH scavenging	Concentration-dependent activity in leaf extracts; colour interference possible	[21, 22]
ABTS and FRAP	Complementary assays covering different mechanisms; FRAP misses hydrogen-transfer activity	[23, 24]
Leaf fractions	Moderately polar fractions, notably ethyl acetate, among the stronger scavengers in vitro	[25]
Leaf, stem and root bark	Leaf, stem bark and root bark extracts differ in polyphenol content and antioxidant behaviour	[26]
Bioavailability of polyphenols	Poor absorption and extensive metabolism limit extrapolation from in vitro assays	[9, 10]

A short note on terminology avoids confusion later. Free radicals are species with an unpaired electron, such as superoxide and hydroxyl radicals, whereas reactive oxygen species also include non-radical molecules like hydrogen peroxide. An antioxidant, in the sense used by Halliwell and Gutteridge, is a substance that at low concentration delays or prevents oxidation of a target [5]. Scavenging a stable synthetic radical in a cuvette is a measure of one property, hydrogen or electron donation, and is not the same as protecting a biological target from oxidative injury. Much of the confusion in the plant antioxidant literature comes from using the word for both ideas without saying which is meant [6, 20].

2. BOTANY AND PHYTOCHEMICAL BACKGROUND

The leaves of Moringa are tripinnate, with small oval leaflets, and are harvested repeatedly from fast-growing trees. Composition varies with cultivar, climate, soil, season and stage of maturity. Siddhuraju and Becker compared leaves from three agroclimatic origins and found differences in phenolic content and antioxidant behaviour among them, a result that has been echoed in later work [16, 18]. This variation is one of

the first things a reader should keep in mind when comparing numbers from different papers, because a sample from West Africa and one from South India may differ in chemistry before any extraction decision has been made.

Nutritional composition helps explain the plant's popularity. Fuglie's widely circulated handbook promoted the leaves as a source of protein, provitamin A, vitamin C, calcium and iron for populations at risk of malnutrition, and later reviews have repeated the claim with varying degrees of care [3, 4]. Nutrient content is not the same as antioxidant content, but the two overlap, since ascorbic acid, carotenoids and tocopherols are antioxidants in their own right. This overlap complicates interpretation. An extract that scores well in a radical scavenging test may owe its result partly to vitamins and only partly to the flavonoids that attract most of the attention [11, 15].

Chemical profiling has identified several families of compounds. Flavonoids, mainly quercetin and kaempferol glycosides, are prominent, along with chlorogenic acids and other phenolic acids [11, 12]. A distinctive group is the glucosinolates and their isothiocyanate breakdown products, including the unusual rhamnose-bearing glucosinolate sometimes called glucomoringin, which Bennett and colleagues described in tissues of the genus [13]. Carotenoids, tocopherols, ascorbic acid, alkaloids and tannins have also been reported [14, 15]. Because the profile depends on the tissue, leaves, pods, roots and seeds are not interchangeable in either their chemistry or their reported activity [11, 26].

Quercetin-3-O-glucoside, rutin and kaempferol glycosides turn up repeatedly in liquid chromatography studies of the leaf. Coppin and colleagues, using liquid chromatography with mass spectrometric detection, quantified a set of flavonoids in leaf extracts and linked them to anti-inflammatory effects in cell models [12]. Amaglo and co-workers compared tissues from trees grown in Ghana and reported substantial amounts of flavonoids, vitamin C and carotenoids in the leaves [11]. These studies matter for screening work, because they tell the analyst which compounds should be expected and which colour reactions are likely to be positive.

3. EXTRACTION AND QUALITATIVE SCREENING

Extraction decides much of what is found. Dried, powdered leaf is usually macerated or percolated with a solvent, and the choice runs from water through ethanol, methanol and acetone to ethyl acetate, hexane or chloroform [27, 28]. Polar solvents extract phenolic glycosides, tannins and many flavonoids more efficiently, while non-polar solvents take up chlorophyll, carotenoids and lipids. Sultana and colleagues showed for several medicinal plants that the extraction solvent and technique altered both yield and antioxidant activity, and aqueous alcohol mixtures often performed better than either pure solvent [17]. Methanol is popular because it extracts efficiently, though its toxicity makes it a poor choice if extracts are to be given to animals.

Other practical details matter as much as the solvent. Sequential extraction from non-polar to polar solvents separates the material into fractions that can be tested separately, whereas a single-solvent extraction gives a complex mixture. Temperature, extraction time, particle size and solvent-to-solid ratio all influence yield, and prolonged heat can degrade labile compounds such as ascorbic acid [27, 29]. Drying method affects composition too, since shade-drying, oven-drying and freeze-drying leave different amounts of phenolics. Papers that fail to report these variables make it hard to reproduce results, and many Moringa studies fall into this category.

Newer extraction technologies have been applied to Moringa and to other leaves. Ultrasound-assisted and microwave-assisted extraction shorten the time needed and often raise phenolic yield compared with simple maceration, because cavitation or rapid heating disrupts cell walls [28, 29]. Supercritical fluids and pressurised solvents are used for specialised purposes. For a teaching or quality control laboratory, however, maceration or Soxhlet extraction remains the standard, and most published activity data derive from these older methods.

That choice has a consequence for comparison: a yield obtained by sonication cannot be set directly against one from overnight cold maceration without allowing for the technique [17, 27].

Qualitative tests follow standard schemes described in pharmacognosy textbooks [30, 31]. The ferric chloride test for phenolics and tannins gives a blue, green or black colour. Alkaline reagent and the Shinoda magnesium and hydrochloric acid test indicate flavonoids. Froth formation on shaking with water suggests saponins, a Liebermann-Burchard reaction hints at steroids and triterpenes, and Mayer or Dragendorff reagents precipitate alkaloids. Leaf extracts of Moringa are most often reported positive for phenolics, flavonoids, tannins, saponins and alkaloids, with the result depending on the solvent employed [14, 15, 26]. Anthraquinones are less consistently found.

These tests are quick and cheap, but they are also non-specific. A positive ferric chloride reaction says only that some phenolic hydroxyl group is present, and the froth test can be fooled by proteins or other surface-active compounds. Alkaloid precipitation reagents are known to give false positives with proteins and some other nitrogenous compounds [30]. Reports that list a long set of positive tests for a single extract, with the term present written beside each class, should therefore be read as a preliminary inventory and not as proof of identity. Thin-layer chromatography, liquid chromatography and spectroscopy are needed to confirm what is actually there.

Pharmacopoeial thinking provides a framework for how screening results should be used. Texts on herbal drug quality control describe macroscopic and microscopic identification, determination of foreign matter, ash values and extractive values, and chromatographic fingerprints as the elements of a quality specification [32]. Qualitative tube tests are a modest part of that scheme. An extract that is screened without any botanical verification of the leaf, or without a record of moisture content and extractive value, cannot be tied to a defined starting material, and the antioxidant result that follows loses much of its value. Good practice starts well before the first reagent is added [30, 31].

Chromatographic fingerprinting adds the specificity that tube tests lack. Thin-layer chromatography with a suitable reference standard such as quercetin gives a quick comparison, and high-performance liquid chromatography with diode array detection can resolve and quantify the main flavonoids and phenolic acids [11, 12]. For a quality control laboratory, a fingerprint that is stable from batch to batch is more useful than a single total phenolic number, and such texts recommend this kind of identity testing alongside macroscopic and microscopic examination [32]. Few antioxidant studies of Moringa extend this far, which weakens the link between the chemistry and the activity they report.

4. COLORIMETRIC QUANTIFICATION OF PHENOLICS AND FLAVONOIDS

Total phenolic content is nearly always measured by the Folin-Ciocalteu method, in which phenolic compounds reduce a phosphomolybdic-phosphotungstic reagent in alkaline conditions to give a blue colour read near 760 nm. The method dates from the 1960s and was later adapted for plant tissues [19, 33]. Results are expressed as gallic acid equivalents, which is a convention and not a statement that the sample contains gallic acid. The reagent is not specific to phenols, and ascorbic acid, reducing sugars and some amino acids also react. Because Moringa leaves contain appreciable ascorbic acid, a high Folin value may overstate the true phenolic content [20, 33].

Total flavonoids are most commonly estimated by an aluminium chloride method, in which aluminium forms an acid-stable complex with certain flavonoids and the absorbance is read in the visible range, with quercetin or rutin as the usual standard [34]. Again, the result is relative to the standard chosen, and a flavonoid lacking the required hydroxyl-keto arrangement may not react at all. The assay is also sensitive to the order of reagent addition and to incubation time. Comparing a value expressed as quercetin equivalents with another expressed as rutin equivalents is not straightforward, and tables

that mix them in a single column can mislead [34, 35].

Reported values for Moringa leaf extracts cover a wide span. Solvent, origin, leaf maturity and the exact protocol all contribute, and it is unusual to see two laboratories agree closely. Siddhuraju and Becker reported high phenolic contents in aqueous methanol and ethanol extracts, and Sreelatha and Padma found differences between leaves at different stages of maturity [16, 18]. Atawodi and colleagues compared methanol extracts of the leaf, stem bark and root bark and reported clear differences in polyphenol content and antioxidant behaviour between the plant parts [26]. These data reinforce a general point: the number matters less than the context in which it was produced.

Sample preparation introduces its own uncertainties. Leaf powder is usually extracted at a fixed ratio of mass to solvent, filtered and concentrated, and the dry residue is redissolved for analysis. Redissolving can be incomplete, particularly for water-poor or lipid-rich extracts, and the insoluble portion may carry active compounds that never reach the cuvette. Chlorophyll absorbs light and may interfere with colorimetric readings, so blanks prepared with the extract but without the reagent are needed [28, 33]. The statistical treatment of replicate measurements, including analysis of variance and the reporting of standard deviations, is often brief. These issues are routine in analytical chemistry, but they are easy to overlook in screening papers.

A useful habit in reading such papers is to ask how the standard curve was prepared, whether extracts were diluted into the linear range, and whether replicate measurements are reported. Those details are often absent. Where a paper reports mean values only, the reader cannot judge variability. Recommendations from IUPAC for antioxidant capacity methods ask for exactly this kind of reporting, along with a statement of the units and the reaction conditions [36]. Methods sections that meet this standard are in the minority for Moringa, though the situation has improved gradually in more recent years.

5. ANTIOXIDANT ASSAYS IN COMMON USE

Antioxidant assays fall broadly into two mechanistic groups. Hydrogen atom transfer methods measure the ability to quench radicals by donating hydrogen, whereas single electron transfer methods measure the ability to reduce an oxidant, with a colour change as the signal. Huang, Ou and Prior set out this distinction clearly, and it remains the framework used in most reviews [20]. Many assays mix both mechanisms in practice, and none reflects the complexity of the cellular environment. Prior and colleagues therefore argued for the use of several complementary assays, together with standardised reporting, instead of a single number [35, 37].

The DPPH assay uses the stable radical 2,2-diphenyl-1-picrylhydrazyl, which is deep purple and fades to yellow as it is reduced. The radical was introduced for antioxidant testing by Blois and the procedure was popularised by Brand-Williams and colleagues [21, 22]. It is cheap, quick and needs only a spectrophotometer, which is why it appears in most Moringa studies. Results are expressed as the concentration giving 50 per cent inhibition (IC50) or as percentage scavenging at one concentration. The radical is soluble in organic solvents and is sterically hindered, so large molecules may react slowly, and strongly coloured extracts can interfere with the absorbance reading [38].

The ABTS or Trolox equivalent antioxidant capacity assay generates a blue-green radical cation that is decolourised by antioxidants, and the improved version by Re and colleagues is the one most often cited [23]. It works in both aqueous and organic media, which makes it suitable for extracts of mixed polarity. The ferric reducing antioxidant power (FRAP) assay of Benzie and Strain measures reduction of a ferric tripyridyltriazine complex to the blue ferrous form at low pH [24]. FRAP does not respond to thiols or to compounds that act by hydrogen atom transfer, so a low result does not mean an extract lacks activity of other kinds [20, 36].

Expression of results varies more than it should. Some papers give percentage inhibition at a single concentration, others

report IC50, and a third group converts activity to equivalents of Trolox, ascorbic acid or gallic acid. A percentage at one concentration is unreliable because the dose-response curve is not linear throughout, and an IC50 depends on the incubation time and on the initial radical concentration [21, 38]. Trolox equivalents bring ABTS and FRAP onto a common scale, but conversion between scales needs care. The IUPAC technical report recommends, wherever feasible, that dose-response curves be shown and that the conditions be spelled out well enough for others to repeat the test [36].

Chemical assays are far removed from biology. None of them considers absorption, metabolism, localisation within cells or the activity of endogenous defences such as superoxide dismutase and glutathione. Prior and Cao compared analytical methods against total antioxidant capacity in vivo and found that the relationship between test-tube numbers and what happens in an organism was weak [37]. For that reason, results from DPPH, ABTS or FRAP are best treated as a ranking tool for extracts under defined conditions, and not as an estimate of health benefit [20, 36]. This view is widely accepted in the field and yet is often forgotten in the discussion sections of individual papers.

Interference deserves a separate mention, because crude plant extracts are far from the clean solutions used to validate methods. Pigments such as chlorophyll and carotenoids absorb in the regions where DPPH and ABTS are read. Turbidity from poorly dissolved material scatters light. Sugars and ascorbic acid reduce the Folin reagent and ferric complexes without being phenolic. Good practice calls for extract blanks, appropriate solvent controls and, ideally, a second assay that works on a different principle [20, 35]. When results from two assays disagree for the same extract, the disagreement is itself informative about mechanism, and it is a mistake to average the two into a single score.

Choice of reference compound also shapes the reading of results. Ascorbic acid, Trolox, gallic acid, butylated hydroxytoluene and quercetin all feature as standards in Moringa papers, and each has a different potency and mechanism. An IC50 for an extract only has meaning relative to the standard run in the same plate. Papers that quote an extract IC50 of a few tens of micrograms per millilitre next to a standard that was not tested are of little help. A pure compound such as quercetin usually outperforms a crude extract on a mass basis simply because the extract is a mixture, and that should not be read as a weakness of the extract [7, 36].

6. REPORTED ACTIVITY OF MORINGA LEAF EXTRACTS

Across published work, leaf extracts consistently show radical scavenging in DPPH, ABTS and reducing power assays, usually in a concentration-dependent manner. Siddhuraju and Becker found that extracts in aqueous alcohols and in 80 per cent methanol performed well in several assays, and that activity tracked total phenolic content [16]. Verma and colleagues reported that some of the more polar fractions of the leaf, notably the ethyl acetate fraction, were among the stronger scavengers, which suggests that moderately polar flavonoids and phenolic acids drive the effect [25]. Sreelatha and Padma observed activity in both mature and tender leaves, with some differences between them [18].

The relationship between phenolic content and antioxidant activity is positive in many reports but not universal. Where a correlation exists, it supports the interpretation that phenolics drive scavenging, as predicted by the structure-activity rules for flavonoids, in which a catechol group in the B ring and a hydroxyl at position 3 favour radical quenching [7]. Where it breaks down, other contributors such as ascorbic acid, carotenoids, tocopherols and glucosinolate-derived compounds may be responsible, or the assay may be responding to non-phenolic reductants [11, 14, 15]. A correlation coefficient from a handful of extracts should not be pushed too far, as it can be strongly affected by a single point.

Solvent effects are reported fairly consistently. Aqueous and alcoholic extracts of the leaf tend to show higher total phenolic content and stronger reducing power than extracts in less polar solvents, which fits the polar nature of flavonoid glycosides and

phenolic acids [16, 17]. Hexane and chloroform extracts, rich in chlorophyll and carotenoids, show weaker scavenging in DPPH but can look better in lipid-based systems. This split illustrates why a single assay cannot rank extracts fairly, and why combining aqueous and lipophilic systems gives a more complete account of the material [20, 36].

Comparison with other plants gives useful perspective. Moringa leaf extracts are frequently described as having high activity, but the phrase is relative, and in studies that include several species the ranking depends on the assay and the solvent. Studies that place Moringa alongside other common leafy vegetables or tea tend to find it comparable to, not far above, plants rich in polyphenols [1, 39]. It is nevertheless striking that a plant eaten as a green vegetable gives strong scavenging activity in both water and alcohol extracts, and that extracts remain active across different harvest stages [18]. Dietary relevance is therefore at least plausible.

The synthesis in table 1 groups published findings by compound class, extraction choice, assay and reported activity, and the distribution of entries is shown in fig. 1. The pattern is informative. Findings about phytochemical classes and about assay behaviour are well represented because they have been studied for decades, whereas entries on bioavailability and in vivo confirmation are relatively few. Anyone planning further work on the species would be wise to put effort where the evidence is thinnest, which means confirmation in biological systems, standardised extracts and identification of the active constituents, not yet another DPPH screen of a different solvent.

Metabolic effects have been investigated more than most other biological activities of the leaf. Mbikay reviewed studies in which leaf preparations lowered blood glucose and improved lipid profiles in rodents, and noted that proposed mechanisms include isothiocyanates, phenolics and alkaloids acting on several pathways [40]. Chumark and colleagues attributed part of the anti-atherosclerotic effect of an aqueous extract in rabbits to antioxidant properties [41]. These results are consistent with the antioxidant story but do not prove it, because the same extract can affect lipid absorption, inflammation and glucose handling by routes unrelated to radical scavenging [14, 42].

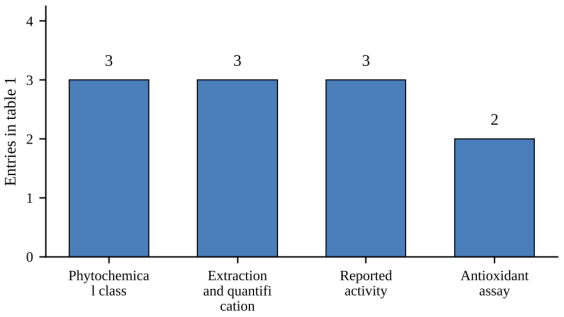


Fig. 1: Number of entries in table 1 by theme

A few reports go beyond chemistry. Chumark and colleagues found that an aqueous leaf extract reduced oxidative markers in animals fed a high-cholesterol diet, linking antioxidant properties to anti-atherosclerotic effects [41]. Vergara-Jimenez and colleagues reviewed bioactive components in the leaves and their possible protection against chronic disease, noting that isothiocyanates and polyphenols may act through pathways beyond direct scavenging, including induction of cytoprotective enzymes [14]. These mechanistic ideas are interesting and still largely preclinical. Human trials, where they exist, are small and heterogeneous, and the review by Stohs and Hartman is a fair summary of the state of play [42].

7. SAFETY AND LIMITATIONS OF THE EVIDENCE

Leaves are generally regarded as safe when eaten in food amounts, and long dietary use supports this. Toxicity data for concentrated extracts are more limited. Stohs and Hartman noted that acute toxicity studies in rodents reported low toxicity for leaf extracts, though root and bark preparations contain alkaloids and other compounds that are of greater concern, particularly in pregnancy [42]. Doses used in antioxidant experiments in vitro have no direct equivalent in humans, and the leaf powder sold as a supplement is not standardised. Anyone interpreting laboratory data for consumer advice should keep these points in view [2, 4].

Polyphenol bioavailability is a further limit. Williamson and Manach reviewed the

evidence from human intervention studies on dietary polyphenols and found that most are poorly absorbed, extensively metabolised in the gut and liver, and present in plasma at low concentrations as conjugates rather than as the parent compounds measured in assays [9]. Scalbert and Williamson drew similar conclusions about intake and absorption [10]. For Moringa, this means the compounds responsible for strong in vitro results may not reach tissues in meaningful amounts. Some effects could arise instead through actions in the gut or through indirect signalling, but this remains hypothetical for the species [8, 9].

Methodological weaknesses are common. Sample identification is often incomplete, with no voucher specimen deposited. Extract yield is not always reported, which prevents conversion of per-gram figures to per-leaf equivalents. Duplicate or triplicate measurements are given without any estimate of between-day variation. Mixed units and standards make cross-study comparison difficult, and some papers treat the lowest IC50 as proof of superiority without statistical testing. IUPAC and other bodies have called for more consistent practice, and the call applies strongly to the Moringa literature [35, 36]. Taken individually these are small faults, but together they explain much of the scatter between laboratories.

For pharmacists and other practitioners, several practical points follow. Customers who ask about Moringa powder or capsules should be told that products differ widely in composition, that the antioxidant claims on labels rest mainly on laboratory assays, and that interaction data are sparse [4, 42]. Leaf preparations contain compounds that might affect blood glucose or blood pressure, so people using antidiabetic or antihypertensive medicines deserve a cautious word of advice [39, 40]. For manufacturers, specifications that include identification, a defined marker content and limits for contaminants such as heavy metals and microbes are a better guarantee of quality than a quoted antioxidant value [32].

8. CONCLUSION

The published literature presents Moringa oleifera leaf extracts as a rich and variable source of flavonoids, phenolic acids, glucosinolate-derived compounds and vitamins, with consistent radical scavenging and reducing activity in standard chemical assays [15, 16, 25]. Solvent, plant origin, maturity and drying all change the outcome, and the colorimetric methods used to quantify phenolics and flavonoids are open to interference from other reducing substances [19, 20, 34]. Taken one by one the findings are plausible, but they are hard to set side by side.

The step from test tube to health benefit remains largely unsupported, because polyphenols are poorly absorbed and heavily metabolised, and human trials are few [9, 42]. Progress will depend on standardised extracts, several complementary assays, chromatographic identification of the active constituents and reporting that follows international recommendations [35, 36]. Until these are in place, antioxidant results for Moringa should be read as a useful screening signal about the extract under defined conditions and not as evidence of therapeutic value.

9. ABBREVIATIONS

DPPH-2,2-Diphenyl-1-picrylhydrazyl, ABTS-2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), FRAP-Ferric reducing antioxidant power, IC50-Concentration giving 50 per cent inhibition, IUPAC-International Union of Pure and Applied Chemistry, WHO-World Health Organization, Trolox-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

10. FUNDING

Nil

11. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

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