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Savan Donga
Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

Sumitra Chanda
Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

Corresponding Author:
Sumitra Chanda
Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

Evaluation of phytochemical profile and antioxidant activity of some medicinal plants seed extracts obtained by traditional and modern (Green) extraction methods

Savan Donga and Sumitra Chanda

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Abstract
The medicinal plants have been one of the major sources of medicines since the beginning of civilization. There is high demand for plant based medicines, nutraceuticals and cosmeceuticals all across the globe. Herbal medicines are becoming more and more popular in recent years with their over increasing acceptability in both developing and developed countries. All parts of the plant leaf, stem, bark, root, flowers, fruits, seeds, peels, are therapeutically useful. But flowers, seeds and peels are less utilized. Seeds are generally thrown into environment and these waste disposal has become a critical global problem. But they have a rich source of many valuable bioactive compounds especially phenols and flavonoids because of which they can be used therapeutically. In the present work, 6 plant seeds viz. *Annona squamosa* L., *Carica papaya* Linn. (Un-ripe), *Carica papaya* Linn. (Ripe), *Ceiba pentandra* L. Gaertn., *Trachyspermum ammi* L. and *Trigonella foenum - graecum* L. were screened for their phytochemical profile and antioxidant efficacy using different extraction techniques. The seeds were extracted by different extraction methods viz. Traditional methods - decoction, maceration and Modern methods - microwave assisted extraction and ultrasonic assisted extraction. The phytochemicals or bioactive components evaluated were total phenols, phenolic acids, flavonoids, flavonols and proanthocyanidin content. *In vitro* antioxidant activity in terms of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, Superoxide anion free radical (O2-) scavenging activity, 2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity and Ferric reducing antioxidant power (FRAP) was evaluated. All the extracts showed varied levels of phytochemical and antioxidant activity. But the best activity was shown by modern methods especially UAE. Thus, seeds can be profitably, beneficially employed as a rich source of bioactive compounds which can be effectively utilized as a source of natural antioxidant and reduce environmental pollution.

Keywords: Seed extracts, extraction techniques, phytochemicals, antioxidant activity, medicinal plants, traditional methods, modern methods

Introduction
Plants are essential part of human civilization. Medicinal plants are relied upon by over 80% of the world population for their necessary health care requirements mainly because they are comparatively cheap, widely easily available and they are free from many side effects associated with synthetic drugs. Diverse plants have been used as a source of novel drugs either in a pure compound form or their extract form and it provided unlimited opportunities to expand a diversity of new innovative drugs (Sen *et al*., 2010) [53].

In traditional Indian medicines, all parts of the plant viz. leaf, stem, bark, root, flowers, fruits, seeds, peel are recognized to have therapeutic properties and have been used to treat various diseases. Any part or rather all parts of the plant are therapeutically useful. However, it is always desirable and beneficial to make use of that part of the plant which is less utilized. For eg. flowers, seeds, peels, etc. All these parts are generally thrown into the environment and this agro or bio waste disposal is problematic economically and environmentally. Huge amount of solid wastes in the form of peels and seeds are generated by the fruit processing industries and these wastes if not disposed properly cause serious environmental problems such as water pollution, unpleasant odors, explosions and combustion, asphyxiation and greenhouse gas emissions.

emissions. But they have a great potential for reuse as antimicrobials, antioxidants, anti-cancer agents, etc. (Mirabella *et al*., 2014; Rakholiya *et al*., 2014; Lee *et al*., 2020) [40, 44, 31].

Infectious diseases and cancer fall under some of the leading cause of deaths worldwide. Now-a-days people are suffering infections caused by multidrug resistant bacteria and fungi. Earlier antibiotic therapy came to the rescue but because of use, misuse and overuse, the antibiotics which were once working or no more able to tackle the microorganisms. Also newer ways of multi drug resistance mechanisms are evolving. Another dire problem is oxidative stress which is because of free radical generation. Free radicals are responsible for a number of diseases and disorders like cancer, atherosclerosis, cardiovascular disease, Parkinson's, liver injury and rheumatoid arthritis (Alok *et al*., 2014) [4]. So in order to tackle these ever green problems, newer alternative drugs are required which will have better efficacy than existing drugs, with a novel mode of action.

Seeds can be used as dietary compounds for eg *Momordica charantia*, *Cucumis sativus*, *Punica granatum* etc. as spices for eg. *Syzygium aromaticum, Piper nigrum, Elettaria cardamomum,* and essential oils can be extracted from them for eg. *Pongamia pinnata* oil, *Mentha piperita* oil, *Azadirachta indica* oil. But some seeds are thrown away into the environment for eg. *Tamarindus indica, Annona squamosa, Ziziphus jujube, Aegle marmelos, Manilkara hexandra,* etc. Seeds have various nutritional compounds and phytoconstituents, they show different activitiesy like *Mesua ferrea* showed antimicrobial activity (Chanda *et al*., 2013) [13], *Prunus persica* seeds showed antioxidant activity (Loizzo *et al*., 2015) [35] while *Cucumis melo* and *Citrullus lanatus* seeds showed analgesic and anti-inflammatory effects (Wahid *et al*., 2020) [61]. There are various phytoconstituents in them especially phenols and flavonoids which show good antioxidant activity. There is a direct correlation between phenol and flavonoid content and antioxidant activity (Vu *et al*., 2017) [59]. It is very well known that plants with good antioxidant activity show various biological activities *Pterocarpus santalinus* leaf, stem and bark - antimicrobial activity (Donga *et al*., 2017) [21], *Cassia spectabilis* leaf - antimalarial activity (Ekasari *et al*., 2018) [22], *Syzygium cumini* leaf - antidiabetic, antioxidant and cytotoxic activities (Artanti *et al*., 2019) [7], *Centella asiatica* - anti-inflammatory activity (Baby *et al*., 2020) [9].

The phytochemical profile of the plant can give us an idea regarding its therapeutic efficacy. The next step is efficient extraction of the phytoconstituents present in them. There are many methods of extraction and they fall into two categories viz. conventional and non-conventional. The former are also known as traditional methods which include infusion, percolation, maceration, reflux extraction, Soxhlet, etc while the later are known as modern or green extraction methods which include high pressure liquid extraction, super critical CO_2 extraction, accelerated solvent extraction, microwave assisted extraction, ultra sonic assisted extraction, Pulsed electric field Extraction, Enzyme assisted extraction etc (Zhang *et al*., 2018) [64]. There are no universal criteria and it varies from plant to plant. Both methods have advantages and disadvantages but the main aim is to use best method of extraction for phenols and flavonoids which will extract completely the phytoconstituents without losing its efficacy and with no modification of its chemical nature.

In the present work, 6 plant seeds viz. *Annona squamosa* L., *Carica papaya* Linn. (Un-ripe), *Carica papaya* Linn. (Ripe), *Ceiba pentandra* L. Gaertn., *Trachyspermum ammi* L. and *Trigonella foenum - graecum* L (Figure 1) were screened for their phytochemical profile and antioxidant efficacy using different extraction techniques. The extraction was done using two traditional methods viz. decoction (DCE) and maceration (MCE) and two modern methods viz. microwave assisted extraction (MAE) and ultrasonic assisted extraction (UAE). *Annona squamosa* L., belongs to Annonaceae family. The seed showed antioxidant and antimicrobial activity (Kothari and Seshadri, 2010 [30]; Gupta *et al*., 2019) [25]. *Carica papaya* Linn. belongs to Cariccaceae family. The seed and latex showed molluscicidal activity (Jaiswal and Singh, 2008) [27] while peel and seed showed antioxidant activity (Ang *et al*., 2012) [6]. *Ceiba pentandra* L. Gaertn., belongs to Bombacaceae family. The seed showed antioxidant activity (Loganayaki *et al*., 2013) [34] while seed oil showed anti-inflammatory activity (Ravi Kiran *et al*., 2014) [48]. *Trachyspermum ammi* L. belongs to Apiaceae family. The seeds showed anticancer and anticandidal activity (Ramya *et al*., 2017; Wahab *et al*., 2020) [46, 60]. *Trigonella foenum - graecum* L belongs to Fabaceae family. The seeds showed antioxidant and antimicrobial (Norziah *et al*., 2015) [42] and antiasthmatic activity (Jain *et al*., 2020) [26].

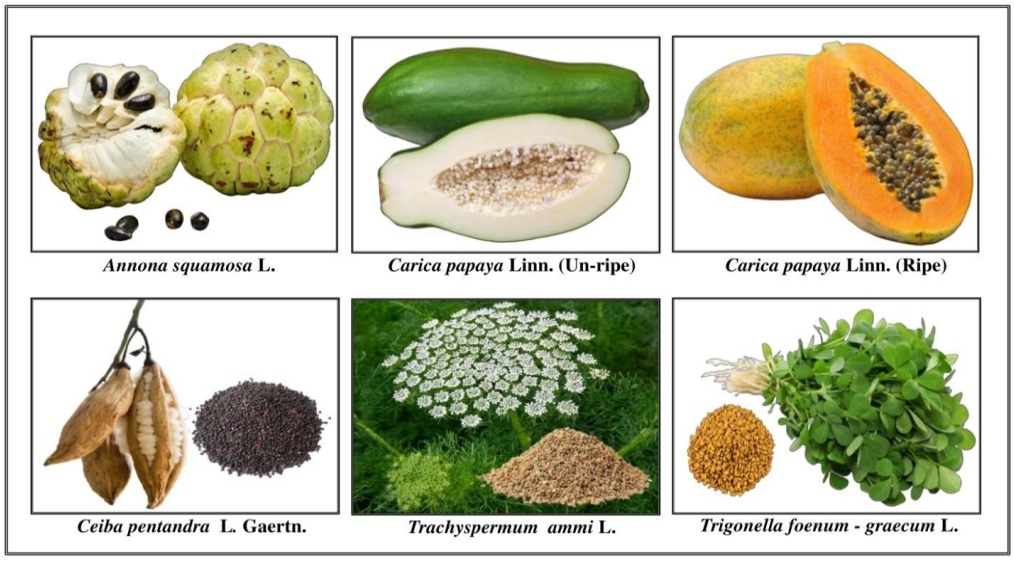


Fig 1: Photographs of screened plant seeds

Considering the above, the aim of the present work was evaluation of phytochemical profile and antioxidant activity of some medicinal plants seed extracts obtained by traditional and modern (green) extraction methods.

Materials and Methods

Plant collection

Trachyspermum ammi L. and *Trigonella foenum - graecum* L were purchased from local market in Rajkot, Gujarat, India while fruits of *Annona squamosa* L., *Carica papaya* Linn. (Un-ripe), *Carica papaya* Linn. (Ripe), *Ceiba pentandra* L. Gaertn., were collected in Rajkot, Gujarat, India. Seeds were separated from fruit, washed with tap water followed by distilled water. The washed seeds were dried under shade. All seeds were crushed to fine powder and stored in air tight closed containers for further studies.

Extraction

The extraction was done using two traditional methods viz. decoction (DCE) and maceration (MCE) (Vongsak *et al.*, 2013) [57] and two modern methods viz. microwave assisted extraction (MAE) (Dahmoune *et al.*, 2015) [18] and ultrasonic assisted extraction (UAE) (Chen *et al.*, 2007) [14]. The procedure followed is as described by Yoganandi *et al.*, (2018) [63].

Quantitative phytochemical analysis and antioxidant activity

The total phenol content (TPC) was determined according to the modified method of Mc Donald *et al*. (2001) [37] by using Folin-Ciocalteu's reagent method. The total phenol content was expressed in terms of gallic acid equivalent (GAE) (mg/g of extracted compound). The phenolic acid content (PAC) of different extracts of *L. bipinnata* leaf was determined according to the modified method of Tomczyk *et al*. (2010) [56] by using Arnov's reagent method. The phenolic acid content was expressed in terms of caffeic acid equivalent (mg /g of extracted compound). The amount of total flavonoid content (TFC) was determined according to the modified method of Boutennoun *et al.,* (2017) [11] by using Aluminium chloride ($AlCL_3$) colorimetric method. The total flavonoid content was expressed in terms of quercetin equivalent (mg/g of extracted compound). The content of flavonols was determined by modified colorimetric method described by Boutennoun *et al.,* (2017) [11] and Abdel-Hameed (2009) [1]. The results are expressed in terms of quercetin equivalent (mg/g of extracted compound). The proanthocyanidin content was determined by the butanol-HCl assay as described by Zilic *et al*. (2011) [65]. The proanthocyanidins content was expressed in terms of leucocyanidin equivalent (mg/g of extracted compound). All samples were analyzed in triplicate and mean values are presented with ± S. E. M (Standard Error of Mean). The procedure followed is as described by Yoganandi *et al.,* (2018) [63]. The antioxidant activity was evaluated by four different *in vitro* antioxidant assays viz. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (Mc Cune and Johns, 2002) [36], Superoxide anion free radical (O_2^-) scavenging activity (Robak and Gryglewski, 1988) [50], 2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity (Re *et al.*, 1999) [49] and Ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996) [10]. The procedure followed is as described by Kaneria *et al*., (2018) [28].

Results and Discussion

Extractive yield

The extractive yield of all 6 plant seeds is given in Fig. 2. The extractive yield was different in different plant seeds; the traditional and modern techniques affected different plant seeds differently. It varied from plant to plant. The trend of extractive yield in different plant seeds was as follows: *A. squamosa* - UAE > DCE > MAE > MCE; *C. papaya* un-ripe - MAE > UAE > MCE > DCE; *C. papaya* ripe - MAE > UAE > DCE > MCE; *C. pentandra* - UAE = MCE = DCE > MAE; *T. ammi* - UAE > MAE > MCE > DCE; *T. foenum – graecum* - MAE > MCE > UAE > DCE. When extractive yield of plant seeds was compared, both the modern techniques MAE and UAE gave maximum extractive yield. In almost all the plant seeds, the traditional techniques DCE or MCE gave lowest extractive yield except in *C. pentandra*. It can be stated that extraction techniques definitely affected the extractive yield in different plant seeds. Maximum extractive yield in all the plant seeds was in the range 15.7% to 31.5% (Fig. 2). Highest extractive yield was in 9 min MAE extract of *T. foenum – graecum* (Fig.2E). Minimum extractive yield in all the plant seeds was in the range 3% to 17.4%. Lowest extractive yield was in MCE extract of *C. papaya* ripe seed (Fig. 2C). Maximum extractive yield in *A. squamosa*, was in 9 min ultra sonicated extracted (26.7%); in *C. papaya* un-ripe seed, it was in 9 min microwaves exposed extract (30.6%); in *C. papaya* ripe seed, it was in 6 min microwaves exposed extract (18.4%); in *C. pentandra*, it was in 6 min ultra sonicated extracted (15.7%); in *T. ammi*, it was in 6 min ultra sonicated extracted (28.4%) and in *T. foenum – graecum*, it was in 9 min microwaves exposed extract (31.5%). When all the 6 plant seeds were compared, maximum extractive yield was in *T. foenum – graecum* plant seed and minimum in *C. papaya* ripe seed (Figs. 2E and 2C).

It is very well reported in the literature that extraction method, extraction conditions and extraction solvents directly influence extractive yield and extractability of bioactive compounds or phytoconstituents (Taddeo *et al*., 2016 [54]; de Oliveira Reis *et al*., 2019) [20]. Pan *et al.*, (2002) [43]. showed that MAE was the best method for the extraction of tanshinones from *Salvia miltiorrhiza*. Dadi *et al.,* (2019) [17] extracted phenols and flavonoids from *Moringa stenopetala* leaves using conventional technique maceration and modern technique UAE and reported that UAE extracts gave significantly higher yield than macerated extracts.

Quantitative Phytochemical Analysis

Total phenol content

The TPC of all 6 plant seeds is given in Fig. 3. The TPC varied with different seeds and with different extraction techniques. The trend of TPC in seeds was as follows: *A. squamosa* - MAE > DCE > MCE > UAE; *C. papaya* un-ripe - UAE > DCE > MAE > MCE; *C. papaya* ripe - MAE > UAE > MCE > DCE; *C. pentandra* - UAE > MCE > MAE > DCE; *T. ammi* - UAE > MCE = DCE > MAE; *T. foenum – graecum* - UAE > MCE > DCE > MAE. Like extractive yield, maximum TPC was in modern extraction techniques UAE and MAE; in 4 plant seeds, UAE extracts had maximum TPC while in 2 plant seeds MAE extracts had maximum TPC. The extraction techniques definitely affected the TPC of all the plant seeds. Maximum TPC of all the plant seeds was in the range 23.3 mg/g to 51.6 mg/g (Fig. 3). Maximum TPC was in 9 min MAE extract of *C. papaya* ripe seed (Fig. 3D). Minimum TPC in all the plant seeds was in the range 11.5 mg/g to 32.2 mg/g. Minimum TPC was in the 9 min UAE

extract of *A. squamosa* plant seed (Fig. 3A). In *A. squamosa*, maximum TPC was in 3 min microwaves exposed extracted (23.3 mg/g); in *C. papaya* un-ripe seed, it was in 9 min ultra sonicated extracted (43.1 mg/g); in *C. papaya* ripe seed, it was in 9 min microwave extracted (51.6 mg/g); in *C. pentandra*, it was in 6 min ultra sonicated extracted (36.4 mg/g); in *T. ammi*, it was in 9 min ultra sonicated extracted (41.9 mg/g) and in *T. foenum – graecum*, it was in 3 min ultra sonicated extracted (30 mg/g). When all the 6 plant seeds were compared, maximum TPC was in *C. papaya* ripe seed and minimum *A. squamosa* plant seed (Figs. 3D and 3A).

Phenolic acid content (PAC)

The PAC of all 6 seeds is given in Fig. 4. PAC varied with different plant seeds and with different extraction techniques. The trend of PAC in different plant seeds was as follows: *A. squamosa* - UAE > MAE = DCE > MCE; *C. papaya* un-ripe - DCE > UAE = MAE > MCE; *C. papaya* ripe - MAE > UAE > DCE > MCE; *C. pentandra* - MAE > DCE > UAE = MCE; *T. ammi* - UAE > MCE > MAE > DCE; *T. foenum – graecum* - MAE > MCE > UAE = DCE. Like extractive yield and TPC, maximum PAC was in modern extraction techniques MAE and UAE. In 3 plant seeds MAE extracts had maximum PAC and in 2 plant seeds, UAE extracts had maximum PAC; while in remaining 1 plant seeds, DCE extracts had maximum PAC. The extraction techniques definitely affected the PAC of all the plant seeds. Maximum PAC of all the plant seeds was in the range 0.06 mg/g to 0.35 mg/g (Fig. 4). Maximum PAC was in 3 min UAE extract of *T. ammi* plant seed (Fig. 4E). Minimum PAC in all the plant seeds was in the range 0.01 mg/g to 0.18 mg/g. Minimum PAC was in the MCE extract of *A. squamosa* plant seed (Fig. 4A) and 9 min MAE extract of *T. foenum graecum* plant seed (Fig. 4F). In *A. squamosa*, maximum PAC was in 3 min ultra sonicated extracted (0.09 mg/g); in in *C. papaya* un-ripe seed, it was in decoction extract (0.08 mg/g); in *C. papaya* ripe seed, it was in 9 min microwaves exposed extract (0.13 mg/g); in *C. pentandra*, it was in 6 min microwave exposed extract (0.16 mg/g); in *T. ammi*, it was in 3 min ultra sonicated extract (0.35 mg/g); in *T. foenum – graecum*, it was in 3 min microwaves exposed extract (0.06 mg/g). When all the 6 plant seeds were compared, maximum PAC was in *T. ammi* plant seed and minimum in *T. foenum – graecum* plant seed (Figs. 4E and 4F).

Total flavonoids content (TFC)

The TFC of all 6 plant seeds is given in Fig. 5. The TFC varied with plant seeds and with different extraction techniques. The trend of TFC in plant seeds was as follows: *A. squamosa* - UAE > MAE > DCE > MCE; *C. papaya* un-ripe - UAE > MAE > DCE > MCE; *C. papaya* ripe - DCE > MCE > UAE > MAE; *C. pentandra* - UAE > DCE > MCE > MAE; *T. ammi* - MAE > UAE > DCE > MCE; *T. foenum – graecum* - UAE > MAE > DCE = MCE. Like extractive yield, TPC and PAC, maximum TFC was in modern extraction techniques UAE and MAE; in 4 plant seeds UAE extracts had maximum TFC and in remaining 2 plant seeds, in 1 plant seed, MAE extracts had maximum TFC and in 1 plant seed, DCE extract had maximum TFC. The extraction techniques definitely affected the TFC of all the plant seeds. Maximum TFC of all the plant seeds was in the range 8.5 mg/g to 14.87 mg/g (Fig. 5). Maximum TFC was in DCE extract of *C. papaya* ripe seed (Fig. 5D). Minimum TFC in all the plant seeds was in the range 0 mg/g to 3.55 mg/g. Minimum TFC was in the MAE extracts of *C. papaya* ripe seed and *C. pentandra* seed (Figs. 5DC and 5B). In fact, in these 2 plant seed MAE extracts, TFC was not detected. In *A. squamosa*, maximum TFC was in 9 min ultra sonicated extracted (11.95 mg/g); in *C. papaya* un-ripe seed, it was in 6 min ultra sonicated extract (8.53 mg/g); in *C. papaya* ripe seed, it was in decoction extract (14.87 mg/g); in *C. pentandra*, it was in 9 min ultra sonicated extract (14.50 mg/g); in *T. ammi*, it was in 9 min microwaves exposed extract (9.20 mg/g) and in *T. foenum – graecum*, it was in 6 min ultra sonicated extract (9.43 mg/g). When all the 6 plant seeds were compared, maximum TFC was in DCE extract of *C. papaya* ripe seed (Fig.5C) and minimum in MAE extracts of *C. papaya* ripe seed and *C. pentandra* seed (Figs. 5D and 5B).

Flavonol content (FC)

The flavonol content of all 6 plant seeds is given in Fig. 6. The FC varied with different plant seeds and with different extraction techniques. The trend of FC in different plant seeds was as follows: *A. squamosa* - UAE > MAE > DCE > MCE; *C. papaya* un-ripe - MAE > UAE > DCE > MCE; *C. papaya* ripe - DCE > MCE > UAE > MAE; *C. pentandra* - MCE > UAE > DCE > MAE; *T. ammi* - MAE > UAE > DCE > MCE; *T. foenum – graecum* - MAE > UAE > MCE > DCE. Like extractive yield, TPC, PAC, TFC maximum FC was in modern extraction techniques MAE and UAE; in 3 plant seeds MAE extracts had maximum FC and in 1 plant seeds, UAE extracts had maximum FC; while in remaining 2 plant seeds, one in DCE extract and other one in MCE extracts. The extraction techniques definitely affected the FC of all the plant seeds. Maximum FC of all the plant seeds was in the range 12.5 mg/g to 43.18 mg/g (Fig. 6). Maximum FC was in DCE extract of *C. papaya* ripe plant seed (Fig. 6D). Minimum FC in all the plant seeds was in the range 0 mg/g to 15.36 mg/g. Minimum FC was in MAE and UAE extracts of *C. papaya* ripe seed (Fig. 6D) and MAE extracts of *C. pentandra* extract (Fig. 6B). In *A. squamosa*, maximum FC was in 9 min ultra sonicated extracted (34.27 mg/g); in *C. papaya* un-ripe seed, it was in 9 min microwaves exposed extract (24.45 mg/g); in *C. papaya* ripe seed, it was in DCE extract (43.18 mg/g); in *C. pentandra*, it was in MCE extract (12.50 mg/g); in *T. ammi*, it was in 9 min microwaves extract (27.18 mg/g) and in *T. foenum – graecum*, it was in 3 min microwaves exposed extract (27.77 mg/g). When all the 6 plant seeds were compared, maximum FC was in *C. papaya* ripe seed (Fig. 6D) and minimum FC was in MAE and UAE extracts of *C. papaya* ripe seed (Fig. 6D) and MAE extracts of *C. pentandra* extract (Fig. 6B).

Proanthocynidin content (PC)

The PC of all 6 plant seeds is given in Fig. 7. The PC varied with plant seeds and with different extraction techniques. The trend of PC in plant seeds was as follows: *A. squamosa* - DCE > UAE > MAE > MCE; *C. papaya* un-ripe - MAE > UAE > DCE > MCE; *C. papaya* ripe - UAE > MAE > MCE > DCE; *C. pentandra* - UAE > MAE > MCE > DCE; *T. ammi* - UAE > DCE > MAE > MCE; *T. foenum – graecum* - UAE > DCE > MAE > MCE. Like extractive yield, TPC, PAC, TFC, FC, maximum PC was in modern extraction techniques UAE and MAE; in 4 plant seeds, UAE extracts had maximum PC and in 1 plant seeds, MAE extracts had maximum PC while in remaining 1 plant seeds, DCE extract showed maximum PC. The extraction techniques definitely affected the PC of all the plant seeds. Maximum PC of all the plant seeds was in the range 0.9 mg/g to 1.7 mg/g Fig. 7. Maximum PC was in 6 min UAE extract of *T. ammi* plant seed (Fig. 7E). Minimum PC in

all the plant seeds was in the range 0 mg/g to 0.7 mg/g. Minimum PC was in the MCE extract of *A. squamosa* plant seed (Fig. 7A). In *A. squamosa*, maximum PC was in DCE extract (1.64 mg/g); in *C. papaya* un-ripe seed, it was in 6 min microwaves exposed extract (1.41 mg/g); in *C. papaya* ripe seed, it was in 9 min ultra sonicated extract (1.30 mg/g); in *C. pentandra*, it was in 3 min ultra sonicated extract (0.99 mg/g); in *T. ammi*, it was in 6 min ultra sonicated extract (1.75 mg/g) and in *T. foenum – graecum*, it was in 9 min ultra sonicated extract (2.43 mg/g). When all the 6 plant seeds were compared, maximum PC was in 6 min UAE extract of *T. ammi* plant seed and minimum in *A. squamosa* plant seed (Figs. 7E and 7A).

Extraction methods greatly influence extraction yield of phenolic compounds and their antioxidant properties and has been studied widely (Das *et al*., 2019) [19]. Several researchers proved that modern methods UAE and MAE gave better yield and bioactive compounds from different plant materials. They also showed better antioxidant activity; though they are some reports where traditional methods were also good or no difference between traditional or modern methods. Rasheed *et al.* (2018) [47] evaluated different extraction methods (decoction, infusion, and maceration) and their effect on the bioactive compound profile of *Hibiscus sabdariffa* extracts; cold maceration was good for extracting anthocyanins and infusion method was good for recovering organic acids from *Hibiscus sabdariffa*. No difference in antioxidant activities of *Propolis* by conventional or modern methods (de Oliveira Reis *et al*., 2019) [20].

Anaya-Esparza *et al.*, (2018) [5] obtained higher phenolic content by UAE than by stirring or thermal decoction from *Justicia spicigera* leaves. Yield, phenols, flavonoids and DPPH scavenging activity was more with non conventional method UAE than with conventional method MCE from peels of different citrus cultivars (Saini *et al*., 2019) [51]. Aguilar-Hernandez *et al*., (2019) [2] used UAE for extracting polyphenols from seed, peel, and columella and pulp from *Annona muricata*. UAE increases extraction efficiency of various phytoconstituents from different plant materials for eg. phenolic compounds, antioxidants, and anthocyanins from *Vitis vinifera* seeds (Ghafoor *et al*., 2009) [24], carnosic acid and rosmarinic acid from *Rosmarinus officinalis* (Ge *et al*., 2012) [23], procyanidins from the *Perilla frutescens* seed hull (Li *et al*., 2019) [32], anthocyanins from cranberries (Klavins *et al*., 2018) [29], flavonoids from *Andrographis echioides* (Ramasamy *et al*., 2019) [45].

MAE has also been successfully employed for extraction of resveratrol from *Polygonum cuspidatum* (Chen, 2013) [15], alkaloids from lotus plumule (Xiong *et al*., 2016) [62], hemicelluloses from lignocellulosic materials (Mihiretu *et al*., 2017) [38], phenol, flavonoid and anthocyanins from *Hibiscus sabdariffa* calyx (Nguyen, 2020) [41], phenols and flavonoids from *Phyllostachys pubescens* shoots (Milani *et al*., 2020) [39]. Li *et al*., 2017) [33] compared traditional methods (Soxhlet extraction and maceration extraction) and modern method MAE for extracting phenols and evaluating antioxidant activity of *Gordonia axillaris* fruit and once again confirmed that modern method MAE gave better results.

Thus in accordance with reported literature, in the present work also, the modern methods MAE and UAE were better for extractive yield and extracting bioactive molecules like phenols, flavonoids, flavonols, phenolic acids and proanthocynidins. MAE and UAE are green extraction methods as compared to traditional techniques and provide shorter extraction time, enhanced extraction efficiency, increased reproducibility, less solvent and energy consumption, simple and prevention of thermo degradation of bioactive compounds (Da Porto & Natolino, 2018 [16]; Savie and Gajic, 2020) [52]. However, for every plant optimization of various parameters involved in the techniques (MAE and UAE) is needed and necessary (Aydar *et al*., 2017 [8], Li *et al*., 2017 [33], Ramasamy *et al*., 2019) [45].

Antioxidant activity

The antioxidant activity was evaluated by four different *in vitro* antioxidant assays. All the extracts, irrespective of the extraction methods showed very poor antioxidant activity. None of the extracts showed DPPH free radical scavenging activity. In almost all the extracts, IC_{50} values were more than 1000 µg/ml. SO activity also showed almost similar trend like that of DPPH. In *C. papaya* ripe and *C. pentandra*, very poor SO activity was found; their IC_{50} values were more than 1000 µg/ml. In *A. squamosa*, *T. ammi* and *T. foenum – graecum* few extracts showed little SO activity while most of the extracts displayed IC_{50} value more than 1000 µg/ml.

All the 6 plant seed extracts showed ABTS cation radical scavenging except *A. squamosa* extracts, but to a varied level. In 2 plants, lowest IC_{50} value was in modern techniques while in 3 plants, it was in traditional techniques (Table 1) clearly indicated that there is no universal criteria and it varies from plant to plant. In *C. papaya* unripe seed extracts, the IC_{50} values ranged from 100.5 – 348 µg/ml and lowest was in maceration extract (100.5 µg/ml); in *C. papaya* ripe seed extracts, the IC_{50} values ranged from 256 - 402 µg/ml and lowest was in 9 min microwaves exposed extract (256 µg/ml); in *C. pentandra* seed extracts, the IC_{50} values ranged from 368 - 656 µg/ml and lowest was in 6 min ultra sonicated extract (368 µg/ml); in *T. ammi* seeds extracts, the IC_{50} values ranged from 70 – 190 µg/ml and lowest was in 9 min ultra sonicated extract (70 µg/ml); in *T. foenum - graecum* seed extracts, the IC_{50} values ranged from 201 - 450 µg/ml and lowest was in decoction extract (201 µg/ml).

The extraction techniques definitely affected the FRAP content of all the plants and there was no general trend; it varied from plant to plant. In *A. squamosa* maximum FRAP content was in 3 min microwaves exposed extract (0.65 M/g) while in *C. papaya* unripe seed it was in 9 min microwaves exposed extract (2.81 M/g); in *C. papaya* ripe seed, it was in 3 min ultra sonicated extract (3.58 M/g); in *C. pentandra*, it was in decoction extract (2.04 M/g); in *T. ammi*, it was in maceration extract (2.25 M/g); in *T. foenum – graecum*, it was in 3 min ultra sonicated extract (1.18 M/g). All the extracts showed FRAP content but amongst all the plants, maximum content was in *C. papaya* ripe seed extract and minimum was in *A. squamosa* seed extract. Overall content was in the order: *C. papaya* ripe seed > *C. papaya* ripe seed > *T. ammi* > *C. pentandra* > *T. foenum – graecum* > *A. squamosa*.

In the present study, weak antioxidant activity was found in all seed extracts extracted by different extraction techniques, which could be the result of the poor solubility of polyphenols and other bioactive molecules in the water extract (Bravo *et al*., 2007) [12]. Similar results i.e. poor antioxidant activities by water extracts in *A. saligna* flowers is reported by Al-Huqail *et al*., (2019) [3]. Tohma *et al*., (2016) [55] reported less antioxidant activity in water extract as compared to ethanol extract in *Salvia* species. However, in some plants water can be used as effective extraction solvent as demonstrated by Vu *et al.*, (2019) [58]; they used water in MAE for extracting phenols and antioxidants from banana peels. It can be concluded that extraction method, extraction conditions and

extraction solvents directly influence extractive yield and extractability of bioactive compounds or phytoconstituents and antioxidant activities in medicinal plants.

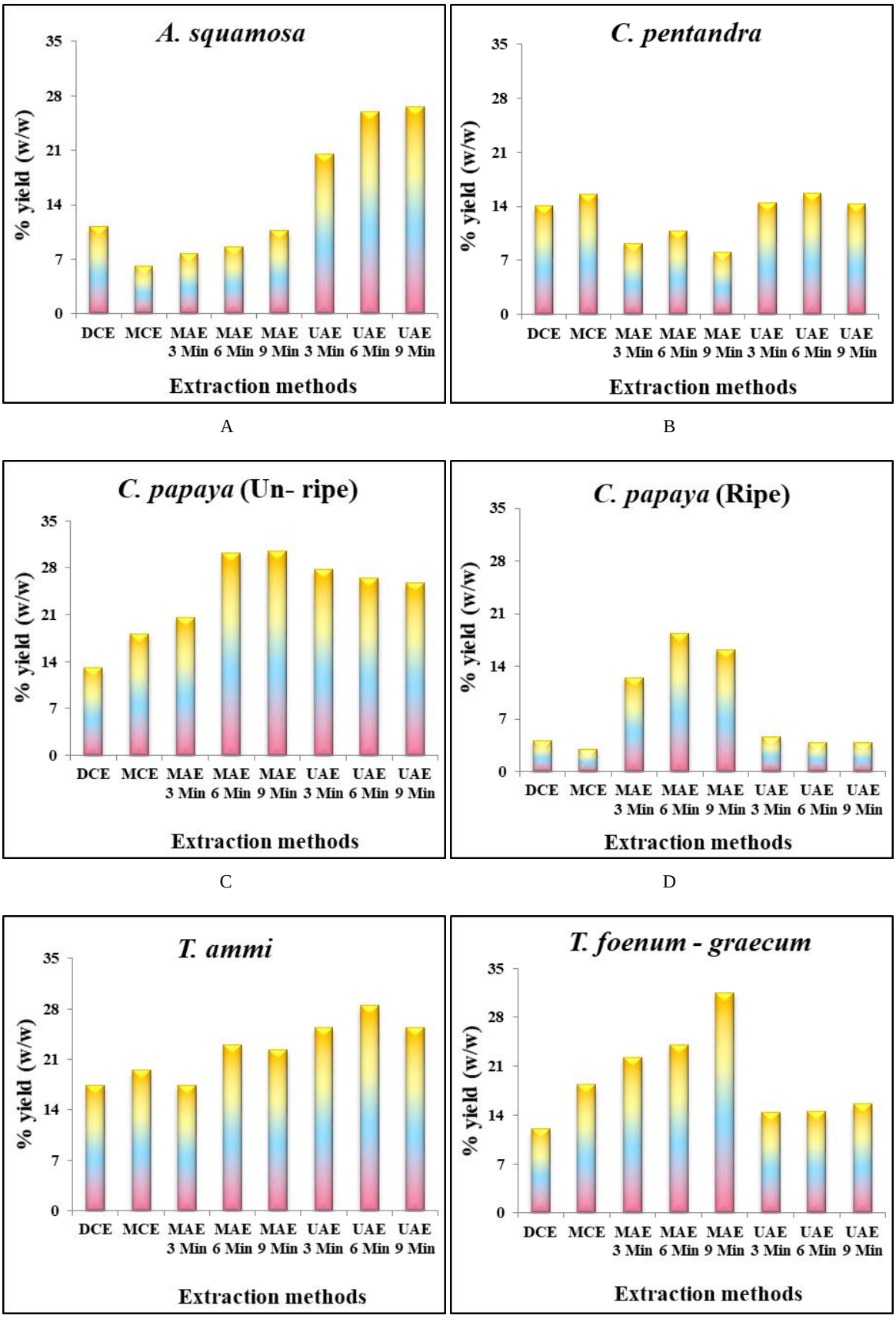


Fig 2: Effect of extraction methods and time on extractive yield of some medicinal plant seeds

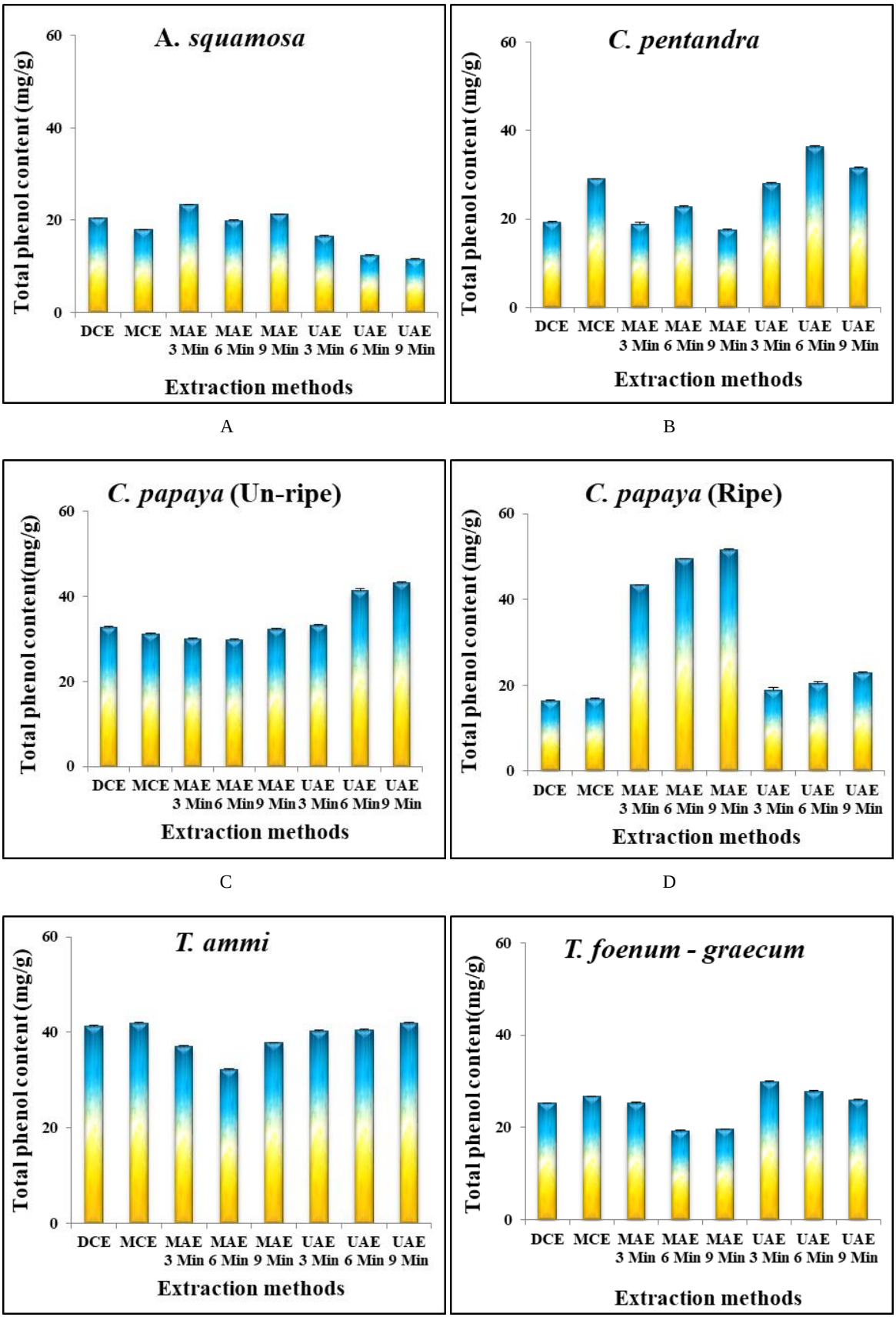


Fig 3: Effect of extraction methods and time on total phenol content of some medicinal plant seeds

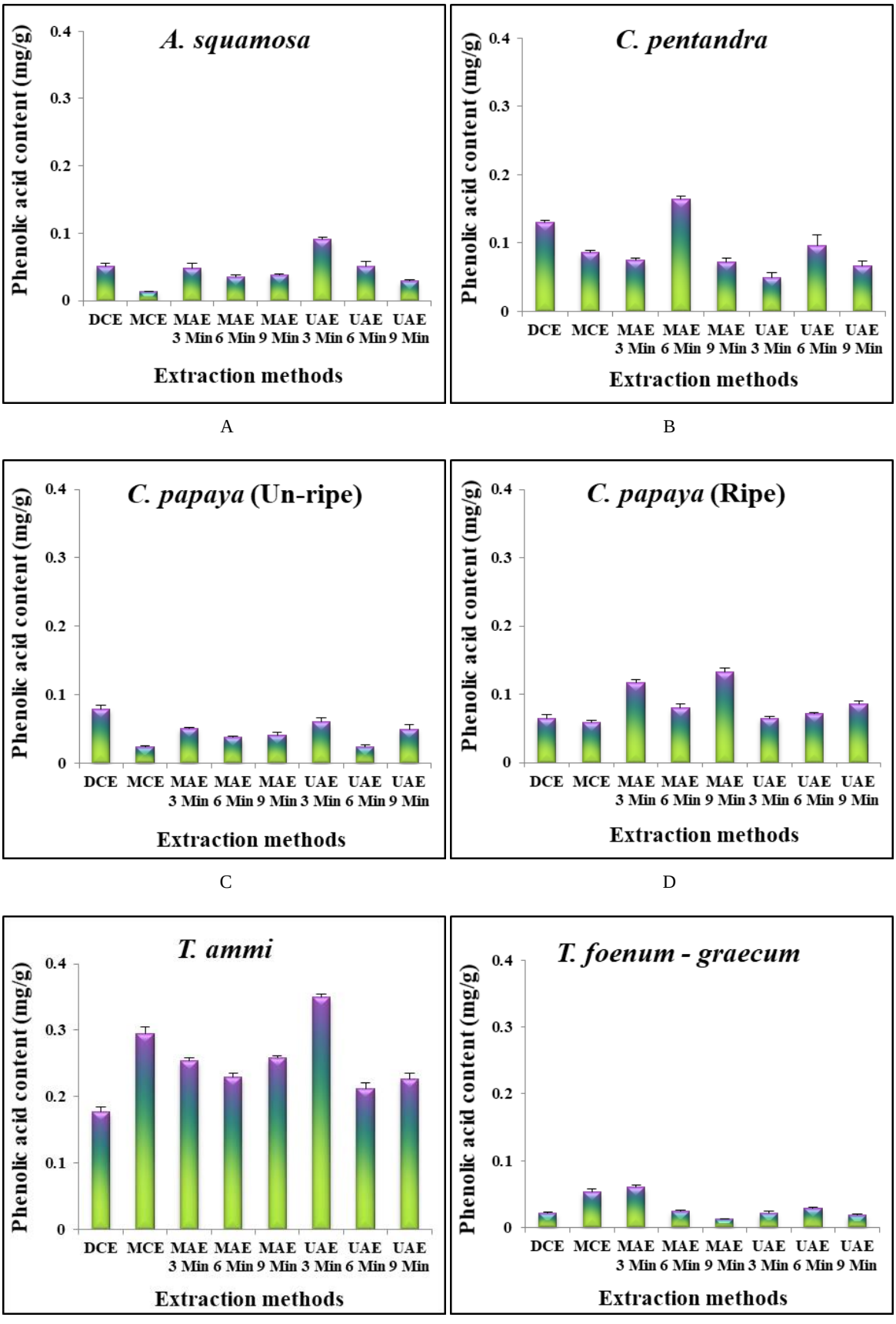


Fig 4: Effect of extraction methods and time on phenolic acid content of some medicinal plant seeds

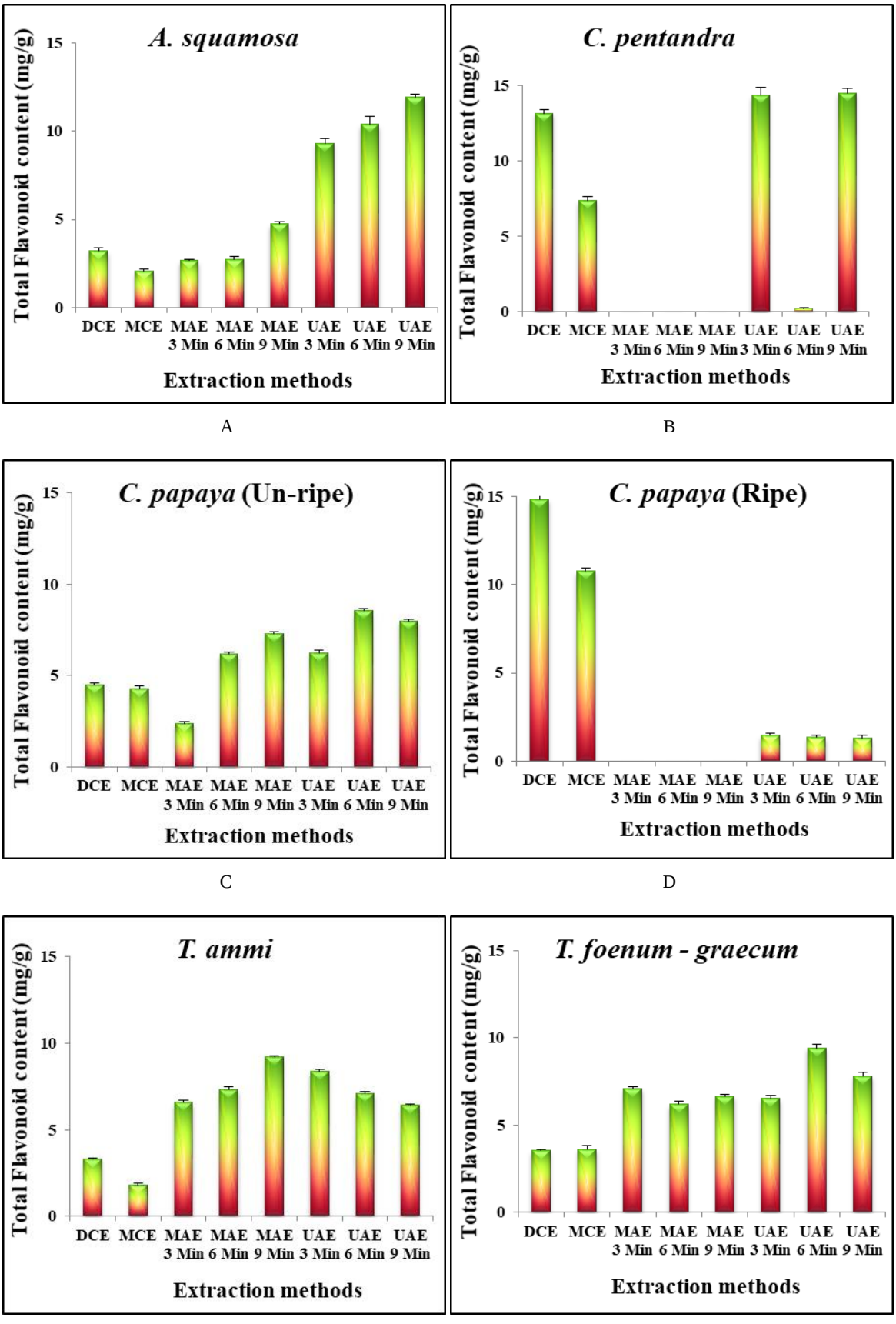


Fig 5: Effect of extraction methods and time on total flavonoid content of some medicinal plant seeds

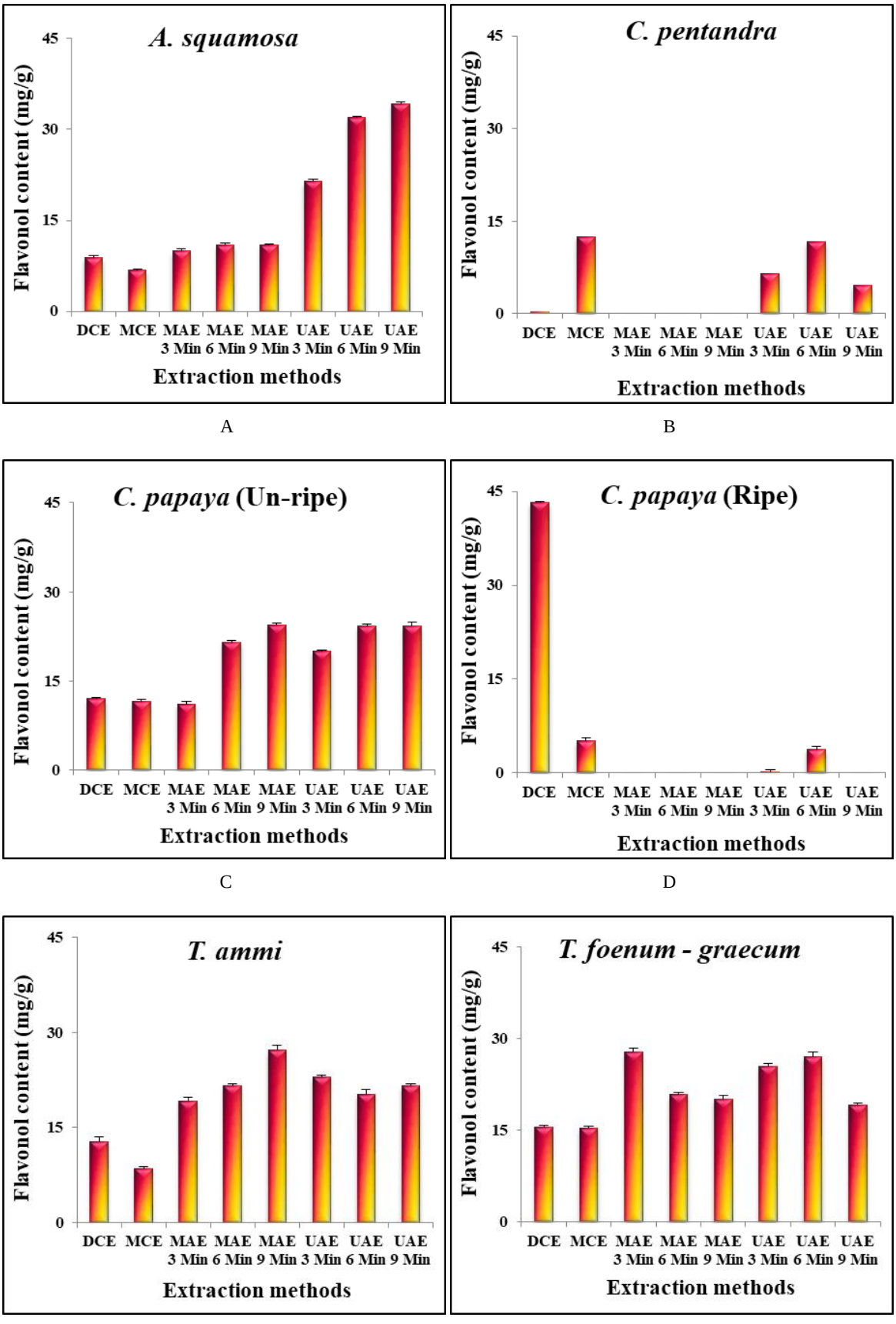


Fig 6: Effect of extraction methods and time on flavonol content of some medicinal plant seeds

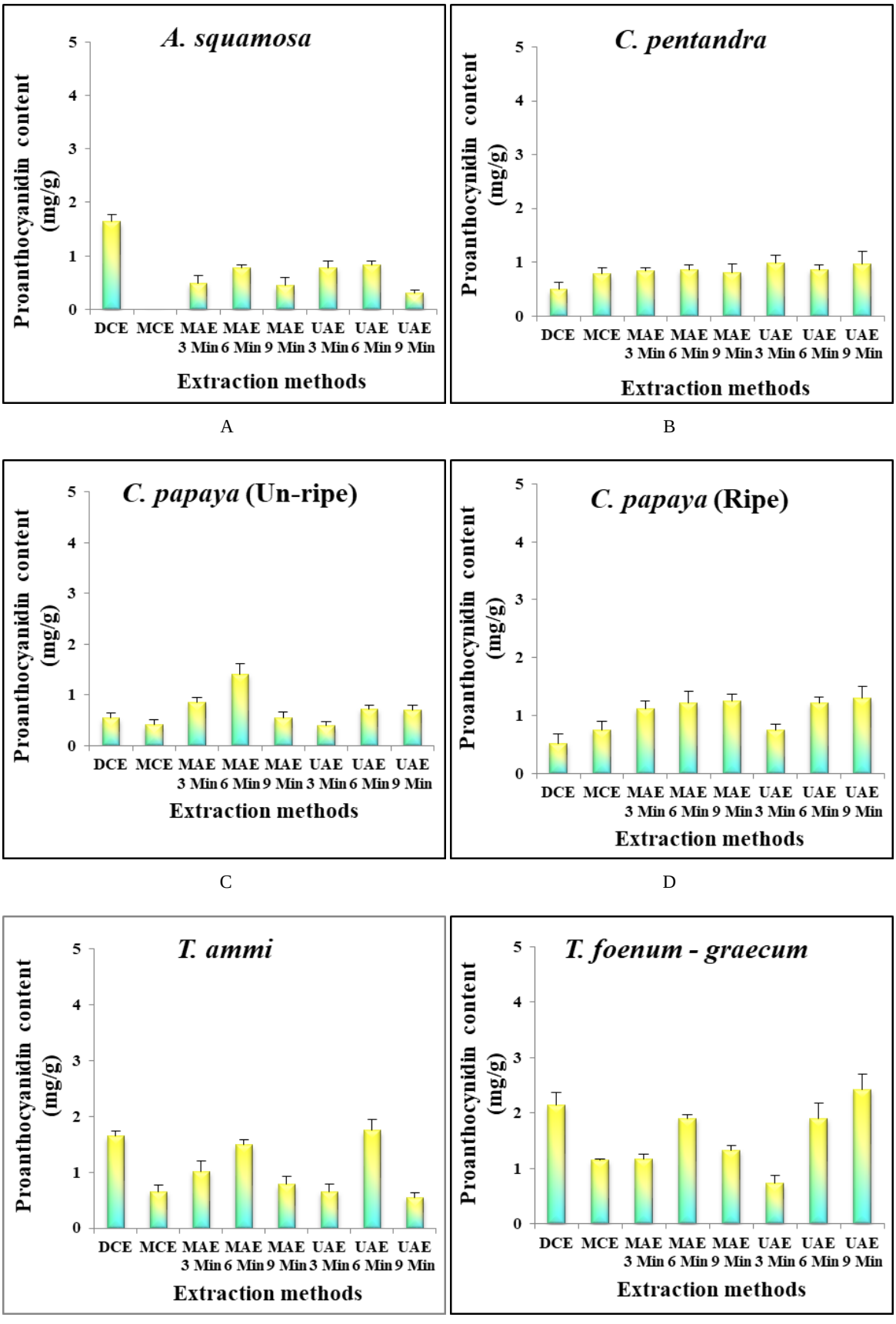
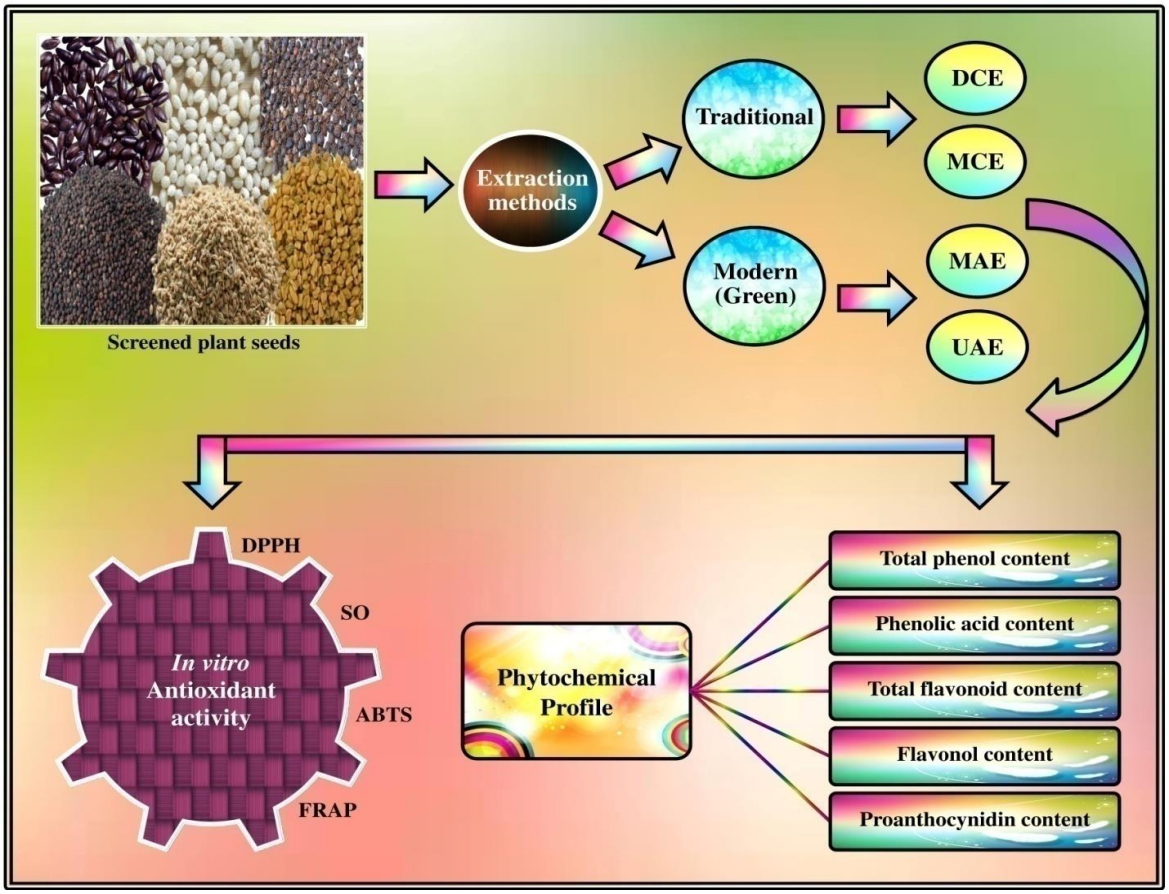


Fig 7: Effect of extraction methods and time on Proanthocyanidin content of some medicinal plant seeds

Table 1: IC_{50} values of DPPH, SO, ABTS and FRAP of different plants seed extracts

Plants Name	Seed extracts		IC_{50} Values(µg/ml)			FRAP (M/g)
			DPPH	SO	ABTS	
A. squamosa	DCE		>1000	>1000	>1000	0.613
	MCE		>1000	360	>1000	0.408
	MAE	3 min	>1000	>1000	720	0.659
		6 min	>1000	>1000	>1000	0.629
		9 min	>1000	384	480	0.645
	UAE	3 min	>1000	>1000	>1000	0.573
		6 min	>1000	>1000	>1000	0.507
		9 min	>1000	>1000	>1000	0.603
C. papaya (Un-ripe)	DCE		>1000	544	228	1.968
	MCE		456	512	100.5	2.645
	MAE	3 min	>1000	504	243	1.549
		6 min	>1000	>1000	348	1.464
		9 min	>1000	>1000	336	2.811
	UAE	3 min	>1000	>1000	207.5	1.939
		6 min	>1000	>1000	195	1.643
		9 min	195	>1000	182.5	2.467
C. papaya (Ripe)	DCE		>1000	>1000	402	2.328
	MCE		>1000	>1000	340	2.016
	MAE	3 min	>1000	>1000	288	2.104
		6 min	824	>1000	324	2.291
		9 min	>1000	>1000	256	2.520
	UAE	3 min	>1000	>1000	390	3.584
		6 min	>1000	>1000	384	2.045
		9 min	>1000	>1000	396	2.707
C. pentandra	DCE		>1000	>1000	656	2.040
	MCE		>1000	>1000	372	1.296
	MAE	3 min	>1000	>1000	495	1.512
		6 min	>1000	>1000	624	1.661
		9 min	>1000	>1000	544	1.112
	UAE	3 min	>1000	>1000	544	1.203
		6 min	>1000	>1000	368	1.816
		9 min	>1000	>1000	512	1.640
T. ammi	DCE		>1000	>1000	190	2.181
	MCE		944	>1000	115.5	2.253
	MAE	3 min	>1000	>1000	140	1.533
		6 min	>1000	>1000	160	1.587
		9 min	>1000	>1000	145	1.547
	UAE	3 min	>1000	>1000	136.5	1.605
		6 min	>1000	592	126	1.736
		9 min	>1000	672	70	1.835
T. foenum – graecum	DCE		>1000	>1000	201	0.824
	MCE		>1000	>1000	216	0.813
	MAE	3 min	>1000	>1000	428	0.720
		6 min	>1000	>1000	284	0.723
		9 min	>1000	>1000	450	0.824
	UAE	3 min	>1000	626	365	1.187
		6 min	>1000	496	426	0.912
		9 min	>1000	>1000	448	0.819



Conclusion: On the basis of the obtained results, it can be concluded that fruit seeds are not waste to be discarded into the environment but they are of some worth and can be therapeutically exploited. They are enriched with phytoconstituents like phenols and flavonoids and hence can be used as a natural source of antioxidants. However, their extractability depends on extraction techniques and extraction solvents and in the present work, modern extraction techniques especially UAE proved best. Antioxidant activity was poor being water extracts and hence extraction by using other organic solvents is desirable. Work in this direction is in progress.

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References

1. Abdel-Hameed ESS. Total phenolic contents and free radicals scavenging activity of certain Egyptian *Ficus* species leaf samples. Food Chemistry. 2009; 114(4):1271-1277.
2. Aguilar-Hernandez G, Garcia-Magana MD, Vivar-Vera MD, Sayago-Ayerdi SG, Sanchez-Burgos JA, Morales-Castro J *et al*. Optimization of ultrasound-assisted extraction of phenolic compounds from *Annona muricata* by-products and pulp. Molecules. 2019; 24(5):904. doi:10.3390/molecules24050904.
3. Al-Huqail AA, Behiry SI, Salem MZ, Ali HM, Siddiqui MH, Salem AZ. Antifungal, antibacterial and antioxidant activities of *Acacia saligna* (Labill.) HL Wendl. flower extract: HPLC analysis of phenolic and flavonoid compounds. Molecules. 2019; 24(4):700. doi:10.3390/molecules24040700.
4. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Herbal antioxidant in clinical practice: A review. Asian Pacific Journal of Tropical Biomedicine. 2014; 4(1):78-84.
5. Anaya-Esparza LM, Ramos-Aguirre D, Zamora-Gasga VM, Yahia E, Montalvo-Gonzalez E. Optimization of ultrasonic-assisted extraction of phenolic compounds from *Justicia spicigera* leaves. The Food Science and Biotechnology. 2018; 27(4):1093-1102.
6. Ang YK, Sia WC, Khoo HE, Yim HS. Antioxidant potential of *Carica papaya* peel and seed. Focusing on Modern Food Industry. 2012; 1(1):11-16.
7. Artanti N, Maryani F, Dewi RT, Handayani S, Dewijanti ID, Meilawati L *et al*. *In vitro* antidiabetic, antioxidant and cytotoxic activities of *Syzygium cumini* fractions from leaves ethanol extract. Indonesian Journal of Cancer Chemoprevention. 2019; 10(1):24-29.
8. Aydar AY, Bagdathiglu N, Koseoglu O. Effect on olive oil extraction and optimization of ultra sound assisted extraction of extra virgin olive oil by response surface methodology (RSM). Grasas y Aceites. 2017, 68(2): doi: http://dx.doi.org/10.3989/ gya.1057162.
9. Baby MS, Antony AV, John B, Jose D. Phytochemical screening and *in vitro* anti-inflamatory activity of ethanolic extract of *Centella asiatica*. World Journal of Current Medical and Pharmaceutical Research. 2020; 2(2):181-183.
10. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power" The FRAP assay. Analytical Biochemistry. 1996; 239:70-76.
11. Boutennoun H, Boussouf L, Rawashdeh A, Al-Qaoud K, Abdelhafez S, Kebieche M *et al*. *In vitro* cytotoxic and antioxidant activities of phenolic components of Algerian *Achillea odorata* leaves. Arabian Journal of Chemistry. 2017; 10(3):403-409.

12. Bravo L, Goya L, Lecumberri E. LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. Food Research International. 2007; 40(3): 393-405.
13. Chanda S, Rakholiya K, Parekh J. Indian medicinal herb: Antimicrobial efficacy of *Mesua ferrea* L. seed extracted in different solvents against infection causing pathogenic strains. Journal of Acute Disease. 2013; 2(4):277-281.
14. Chen F, Sun Y, Zhao G, Liao X, Wu J, Wang Z. Optimization of ultrasound assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography-mass spectrometry. Ultrasonic Sonochemistry. 2007; 14(6):767-778.
15. Chen H. Optimization of microwave-assisted extraction of resveratrol from *Polygonum cuspidatum* sieb et Zucc by orthogonal experiment. Natural Products: An Indian Journal. 2013; 9(4):138-142.
16. Da Porto C, Natolino A. Extraction kinetic modelling of total polyphenols and total anthocyanins from saffron floral bio-residues: Comparison of extraction methods. Food Chemistry. 2018; 258:137-143.
17. Dadi DW, Emire SA, Hagos AD, Eun JB. Effect of ultrasound-assisted extraction of *Moringa stenopetala* leaves on bioactive compounds and their antioxidant activity. Food Technology and Biotechnology. 2019; 57(1): 77-86.
18. Dahmoune F, Nayak B, Moussi K, Remini H, Madani K. Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chemistry. 2015; 166: 585-595.
19. Das S, Ray A, Nasim N, Nayak S, Mohanty S. Effect of different extraction techniques on total phenolic and flavonoid contents and antioxidant activity of betelvine and quantification of its phenolic constituents by validated HPTLC method. 3 Biotech 9. 2019; 9(1):37.
20. de Oliveira Reis JH, de Abreu Barreto G, Cerqueira JC, dos Anjos JP, Andrade LN *et al*. Evaluation of the antioxidant profile and cytotoxic activity of red propolis extracts from different regions of northeastern Brazil obtained by conventional and ultrasound-assisted extraction. Plos one. 2019, 14(7). https://doi.org/10.1371/journal.pone.0219063.
21. Donga S, Moteriya P, Chanda S. Evaluation of antimicrobial and synergistic antimicrobial properties of *Pterocarpus santalinus*. Asian Journal of Pharmaceutical and Clinical Research. 2017; 10(11):204-209.
22. Ekasari W, Wahyuni TS, Arwaty H, Putri NT. Determination of effective dose of antimalarial from *Cassia spectabilis* leaf ethanol extract in plasmodium berghei-infected mice. African Journal of Infectious Diseases. 2018; 12(1S):110-115.
23. Ge Zu, Rongrui Zhang, Lei Yang, Chunhui Ma, Yuangang Zu, Wenjie Wang. Ultrasound-assisted extraction of carnosic acid and rosmarinic acid using ionic liquid solution from *Rosmarinus officinalis*. International Journal of Molecular Sciences. 2012; 13:11027-11043.
24. Ghafoor K, Choi YH, Jeon JY, Jo IH. Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants and anthocyanins from grape (*Vitis vinifera*) seeds. Journal of Agricultural and Food Chemistry. 2009; 57: 4988e4994.
25. Gupta SP, Singh MR, Ahemad I. Evaluation of antimicrobial activity of seed extracts of *Annona squamosa* L. and *Azadirachta indica*. Journal of Medicinal Plants. 2019; 7(4): 282-284.
26. Jain PG, Patil PP, Patil SD, Patil SD, Surana SJ. Evaluation of the antiasthmatic activity of methanolic extract of *Trigonella Foenum Graecum* on experimental models of bronchial asthma. Journal of Drug Delivery and Therapeutics. 2020; 10(1):101-106.
27. Jaiswal P, Singh DK. Molluscicidal activity of *Carica papaya* and *Areca catechu* against the freshwater snail *Lymnaea acuminata*. Veterinary Parasitology. 2008; 152:264-270.
28. Kaneria MJ, Rakholiya KD, Marsonia LR, Dave RA, Golakiya BA. Nontargeted metabolomics approach to determine metabolites profile and antioxidant study of Tropical Almond (*Terminalia catappa* L.) fruit peels using GC-QTOF-MS and LC-QTOF-MS. Journal of Pharmaceutical and Biomedical Analysis. 2018; 160:415-427.
29. Klavins L, Kviesis J, Nakurte I, Klavins M. Berry press residues as a valuable source of polyphenolics: Extraction optimisation and analysis. LWT- Food Science and Technology. 2018; 93:583-591.
30. Kothari V, Seshadri S. Antioxidant activity of seed extracts of *Annona squamosa* and *Carica papaya*. Nutrition & Food Science. 2010; 40(4). doi:10.1108/00346651011062050.
31. Lee JJ, Saiful YL, Kassim NK, Che Abdullah CA, Esa N, Lim PC *et al*. Cytotoxic activity of *Christia vespertilionis* root and leaf extracts and fractions against breast cancer cell lines. Molecules. 2020; 25: doi:10.3390/ molecules25112610.
32. Li HZ Tan YL, Zhang ZJ, Xia YY, Li XJ, Cui LX, Chen T. Optimization of ultrasound-assisted extraction of procyanidins from perilla seed hull and their antioxidant activities *in vitro*. Food Science and Technology. 2019; 39(2):378-387.
33. Li Y, Li S, Lin SJ, Zhang JJ, Zhao CN, Li HB. Microwave-assisted extraction of natural antioxidants from the exotic *Gordonia axillaris* fruit: Optimization and identification of phenolic compounds. Molecules. 2017; 22(9):1481. doi:10.3390/molecules22091481.
34. Loganayaki N, Siddhuraju P, Manian S. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. Journal of Food Science and Technology. 2013; 50(4):687-695.
35. Loizzo MR, Pacetti D, Lucci P, Nunez O, Menichini F, Frega NG *et al*. *Prunus persica* var. *platycarpa* (Tabacchiera Peach): Bioactive compounds and antioxidant activity of pulp, peel and seed ethanolic extracts. Plant Foods for Human Nutrition. 2015; 70(3):331-337.
36. Mc Cune L, Johns T. Antioxidant activity in medicinal plant associated with the symptoms of diabetes mellitus used by the indigenous people of the North American boreal forest. Journal of Ethanopharmacology. 2002; 82:197-205.
37. Mc Donald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chemistry. 2001; 73(1): 73-84.
38. Mihiretu GT, Brodin M, Chimphango AF, Oyaas K, Hoff BH, Gorgens JF. Single-step microwave-assisted hot water extraction of hemicelluloses from selected

lignocellulosic materials - A biorefinery approach. Bioresource Technology. 2017; 241:669-680.
39. Milani G, Curci F, Cavalluzzi MM, Crupi P, Pisano I, Lentini G *et al*. Optimization of microwave-assisted extraction of antioxidants from bamboo shoots of *Phyllostachys pubescens*. Molecules. 2020; 25(1):215. doi:10.3390/molecules25010215.
40. Mirabella N, Castellani V, Sala S. Current options for the valorization of food manufacturing waste: A review. Journal of Cleaner Production. 2014; 65:28-41.
41. Nguyen MP. Mircowave-assisted extraction of phytochemical constituents in roselle (*Hibiscus sabdariffa* L.). Journal of Pharmaceutical Research International. 2020; 32(2):1-12.
42. Norziah MH, Fezea FA, Bhat R, Ahmad M. Effect of extraction solvents on antioxidant and antimicrobial properties of fenugreek seeds (*Trigonella foenum-graecum* L.). International Food Research Journal. 2015; 22(3):1261-1271.
43. Pan X, Niu G, Liu H. Comparison of microwave-assisted extraction and conventional extraction techniques for the extraction of tanshinones from *Salvia miltiorrhiza* bunge. Biochemical Engineering Journal. 2002; 12(1):71-77.
44. Rakholiya K, Kaneria M, Chanda S. Inhibition of microbial pathogens using fruit and vegetable peel extracts. International Journal of Food Science and Nutrition. 2014; 65(6):733-739.
45. Ramasamy J, Kandasamy R, Palanisamy S, Nadesan S. Optimization of ultrasonic-assisted extraction of flavonoids and anti-oxidant capacity from the whole plant of *Andrographis echioides* (L.) nees by response surface methodology and chemical composition analysis. Pharmacognosy Magazine. 2019; 15(65):547-556.
46. Ramya N, Priyadharshini, Prakash R, Dhivya R. Anti cancer activity of *Trachyspermum ammi* against MCF-7 cell lines mediates by p53 and Bcl-2 mRNA levels. The Journal of Phytopharmacology. 2017; 6(2):78-83.
47. Rasheed DM, Porzel A, Frolov A, El Seedi HR, Wessjohann LA, Farag MA. Comparative analysis of *Hibiscus sabdariffa* (roselle) hot and cold extracts in respect to their potential for α-glucosidase inhibition. Food Chemistry. 2018; 250:236-244.
48. Ravi Kiran C and Raghava RT. Lipid profiling by GC-MS and anti-inflammatory activities of *Ceiba pentandra* seed oil. Journal of Biologically Active Products from Nature. 2014; 4(1):62-70.
49. Re R, Pellegrini N, Proteggentle A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolourisation assay. Free Radical Biology & Medicine. 1999; 26:1231-1237.
50. Robak J, Gryglewski RJ. Flavonoids are scavenger of superoxide anions. Biochemical Pharmacology. 1988; 37:837-841.
51. Saini A, Panesar PS, Bera MB. Comparative study on the extraction and quantification of polyphenols from citrus peels using maceration and ultrasonic technique. Current Research in Nutrition and Food Science. 2019; 7(3):678-685.
52. Savic IM, Gajic IM. Optimization of ultrasound-assisted extraction of polyphenols from wheatgrass (*Triticum aestivum* L.). Journal of Food Science and Technology. 2020; 57(8):2809–2818.
53. Sen K, Goel A, Rawal S, Mahajan N, Baboo S, Alok S. Antimicrobial activity of *Basella rubra* leaves. International Journal of Pharmaceutical Sciences and Research. 2010; 1(2):88-91.
54. Taddeo VA, Epifano F, Fiorito S, Genovese S. Comparison of different extraction methods and HPLC quantification of prenylated and unprenylated phenylpropanoids in raw Italian propolis. Journal of Pharmaceutical and Biomedical Analysis. 2016; 129:219-223.
55. Tohma H, Koksal E, Kılıc O, Alan Y, Yılmaz MA, Gulcin I *et al*. RP-HPLC/MS/MS analysis of the phenolic compounds, antioxidant and antimicrobial activities of *Salvia* L. Species. Antioxidants. 2016; 5(4):38. doi:10.3390/antiox5040038.
56. Tomczyk M, Pleszczynska M, Wiater A. Variation in total polyphenolics contents of aerial parts of *Potentilla* species and their anticariogenic activity. Molecules. 2010; 15: 4639-4651.
57. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Industrial Crops and Products. 2013; 44:566-571.
58. Vu HT, Scarlett CJ, Vuong QV. Maximising recovery of phenolic compounds and antioxidant properties from banana peel using microwave assisted extraction and water. Journal of Food Science and Technology. 2019; 56(3): 1360-1370.
59. Vu HT, Scarlett CJ, Vuong QV. Optimization of ultrasound-assisted extraction conditions for recovery of phenolic compounds and antioxidant capacity from banana (*Musa cavendish*) peel. Journal of Food Processing and Preservation. 2017; 41:e13148.
60. Wahab AT, Ilyas Q, Farooq S, Javaid S, Ahmed S, Rahman AU *et al*. *In-vitro* and *in-vivo* anticandidal activity of *Trachyspermum ammi* (L.) sprague seeds ethanolic extract and thymol-containing hexanes fraction. Natural Product Research. 2020; https://doi.org/10.1080/14786419.2020.1731738.
61. Wahid S, Khan RA, Zeeshan F, Ikram R. Analgesic, anti-inflammatory and toxic effects of ethanol extracts of *Cucumis melo* and *Citrullus lanatus* seeds. Pakistan Journal of Pharmaceutical Sciences. 2020; 33:1049-1055.
62. Xiong W, Chen X, Lv G, Hu D, Zhao J, Li S. Optimization of microwave assisted extraction of bioactive alkaloids from lotus plumule using response surface methodology. Journal of Pharmaceutical Analysis. 2016; 6(6):382-388.
63. Yoganandi J, Pande J, Donga S, Chanda S. Comparative evaluation of different extraction techniques on various phytoconstituents of *Terminalia crenulata* Roth. Research Trends in Medicinal Plant Sciences. 2018; 3:121-149.
64. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine. 2018; 13(1):20. https://doi.org/10.1186/ s13020-018-0177-x.
65. Zilic S, Sukalovic VHT, Dodig D, Maksimovic V, Maksimovic M, Basic Z. Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. Journal of Cereal Science. 2011; 54(3):417-424.